NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS

RESPONSE TO THE QUESTIONS POSED BY THE FOOD AND DRUG ADMINISTRATION, THE FOOD SAFETY AND INSPECTION SERVICE, THE CENTERS FOR DISEASE CONTROL AND PREVENTION, THE NATIONAL MARINE FISHERIES SERVICE, AND THE DEPARTMENT OF DEFENSE VETERINARY SERVICE ACTIVITY REGARDING CONTROL STRATEGIES FOR REDUCING FOODBORNE NOROVIRUS INFECTIONS

NOVEMBER 17, 2014

Executive Summary

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF or Committee) was asked to assess the incidence and public health burden of human norovirus (HuNoV) infection and the importance of food as a source of attribution to foodborne HuNoV infections. The Committee was also asked to provide advice on intervention, control, and mitigation strategies to reduce, inactivate and/or eliminate HuNoV contamination in foods, on surfaces and in the environment; to evaluate the current methods for detection of HuNoV in food and environmental samples; and to determine what data are still needed to conduct a quantitative risk assessment. Individual working groups were developed to address the charge questions. Each chapter outlines the Committee response to the charge questions as well as gaps and recommendations for additional data and/or research needs. Summary recommendations of the Committee are identified at the end of the document.

In the background statement relative to the charges it was noted that HuNoVs have been identified as the leading cause of both acute gastroenteritis and foodborne infection in the United States, as well as a leading cause of epidemic and sporadic acute gastroenteritis (AGE) worldwide. However, the fraction of illness attributable to food has been estimated using outbreak data, which indicates a greater tendency to investigate and report outbreaks as foodborne rather than person-to-person. The Committee concluded that there are existing gaps in the data related to transmission and secondary transmission of the illness. After examining the current scientific and technical literature, the Committee determined that, while there are many publications related to noroviruses, and several research groups are currently investigating norovirus, the current methods for detection of HuNov have significant limitations. The lack of sensitive and specific methods to rapidly detect infectious virus in clinical and food samples was identified as a major barrier to advancing the understanding of HuNoV foodborne ecology, epidemiology, transmission, control and detection. The Committee agreed with currently existing guidance that address norovirus prevention, control and outbreak management by: excluding ill food workers; limiting bare-hand contact with ready-to-eat foods; practicing proper hand hygiene; and cleaning and sanitizing surfaces on a frequent basis. However, a significant amount of research related to additional interventions, mitigation and control strategies has been conducted using surrogate organisms. A lack of comparative data between surrogates and HuNoV raises questions on the appropriateness of surrogates as models for HuNoV behavior. Therefore, the Committee was unable to assess the most appropriate control strategy or intervention for use at this time and recommended that surrogates need to be identified and confirmed as adequate representatives of HuNoVs for use in research, validation and verification.

Chapter 1. INTRODUCTION: Statement of Charge to NACMCF and the Rationale for the Approach to Address the Charge

1.1. Charge to the Committee:

Control Strategies for Reducing Foodborne Norovirus Infections

Executive Summary of the Charge:

Many viruses can be transmitted by contaminated foods and subsequently cause disease in humans. Human noroviruses (HuNoVs) are now recognized as the leading cause of foodborne disease in much of the developed world. The Food Safety and Inspection Service (FSIS), the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), the National Marine Fisheries Service (NMFS), and the Department of Defense Veterinary Service Activity (DoDVSA) believe a unified approach to reducing illness from HuNoVs is essential. These agencies issue this charge to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF or Committee) because:

- HuNoVs are the most commonly identified cause of foodborne outbreaks in the United States;
- information gaps exist for the overall burden of disease, different transmission pathways, and most importantly, contamination routes; and, control of HuNoVs transmission through food is critical to public health

Background*:

HuNoVs are the leading cause of epidemic and sporadic acute gastroenteritis (AGE) worldwide and the leading cause of both gastroenteritis and foodborne infection in the United States. This group of related viruses typically causes acute nausea, vomiting and watery diarrhea accompanied by abdominal cramping, and sometimes low-grade fever and malaise. Treatment is supportive to prevent or manage dehydration, and symptoms usually resolve within 1-3 days in otherwise healthy individuals. Some illnesses lead to dehydration, hospitalization and death, particularly among the elderly or immunocompromised. Stools and vomitus are highly infectious, and the infectious dose appears to be extremely low. The virus is easily spread directly from one person to another by contact, through contaminated food and water, via contaminated fomites, and through aerosolized vomitus.
HuNoVs, and the less prevalent, but related human sapoviruses, are members of the *Caliciviridae* family. The first norovirus to be recognized was the "Norwalk agent", identified in

* Parts of the background, outbreaks, and food relationship sections have been updated from the original charge. The charge questions listed above are specifically related to the 2009 edition of the FDA Food Code, hereafter referred to as the Food Code (US FDA, 2009). While the Committee took into consideration the more recently published 2013 edition of the Food Code (US FDA Food Code, 2013) in answering these questions, no changes have been made in this updated version that apply to the original questions posed.

1972 after an outbreak of gastroenteritis in Norwalk, Ohio in 1968 (Adler 1969; Kapikian 1972). Other closely related viruses were called "small round structured viruses", or "Norwalk-like viruses", until the sequence of the complete Norwalk virus genome led to the grouping of these viruses into a new genus *Norovirus* within the family *Caliciviridae* (ICTV 2005).

Outbreaks*:

Among norovirus outbreaks reported to the CDC National Outbreak Reporting System (NORS) between 2009 and 2012, 69% were person-to-person, 23% were foodborne, 0.4% were environmental, and 0.3% were waterborne; the mode of transmission was undetermined for 7% (Hall et al, 2014). In other recently collected data, among 2,895 norovirus outbreaks with routes of transmission reported to CaliciNet, a national norovirus outbreak surveillance network, coordinated by CDC, between 2009 and 2013, 84% were person-to-person, and 16% were foodborne (Vega et al, 2014). Reports to NORS represent fully investigated outbreaks; however, the NORS data may be biased to include more foodborne outbreaks compared to other types of outbreaks that are reported through CaliciNet. The HuNoV foodborne outbreaks reported in 2009-2012 were 48% of reported foodborne outbreaks with known etiology (Hall et al, 2014). Among the non-foodborne outbreaks for which location was reported, 80% were in long-term care facilities, and another 4% were in hospitals; only 1% occurred in restaurant settings. However, among the foodborne norovirus outbreaks reported to CDC, 81% occurred in commercial food service settings, 4% in private settings, 2% in institutional settings; only 1% occurred in long-term care facilities. These data help to characterize the relative importance of different HuNoV transmission routes, but more information is necessary to understand the multiple routes of HuNoV transmission and their association with different types of facilities where food is prepared or served.

Food Relationship*:

The food vehicles most commonly associated with foodborne HuNoV outbreaks include molluscan shellfish, sandwiches, salads, and fresh produce. Dairy products and delicatessen meat have also been implicated in outbreaks. Data collected by the CDC and recent publications indicated HuNoVs are the most common microbial hazards linked to outbreaks associated with fresh produce (i.e., fresh fruits, vegetables, and salads). CDC estimates that 36% of foodborne infections due to HuNoV are attributed to leafy greens (Painter, 2013). Although foods are most often contaminated at the point of preparation or service, HuNoVs may be introduced to food vehicles further up the food distribution chain, such as during production, harvest, or processing.

The 2009 FDA Food Code (US FDA, 2009) specifies preventive controls to reduce the survival and transmission of pathogens in foods prepared and sold in retail and foodservice settings. Many of these preventive controls are developed to address bacterial pathogens rather than viral pathogens such as HuNoV. These preventive controls become regulatory mandates for the retail food industry when adopted by state, local, and tribal authorities that license and inspect these facilities. Since the first edition of the Food Code in 1993, requirements that address the control of HuNoVs and other pathogens have been reviewed and updated as scientific information and best practices have advanced.

Charge Questions:

The epidemiological evidence clearly indicates that HuNoV infection is both common and difficult to control. Progress in reducing foodborne transmission of HuNoVs requires careful review of our current understanding of its epidemiological significance, transmission, and control. The specific charge to the Committee is to consider the following questions during its deliberations:

1) What is known about the incidence and public health burden of HuNoV infection in the United States and in other developed countries? Are there population subgroups at increased risk of more severe disease? Please consider in your response:

   - the value of improvements in compliance with the CDC National Outbreak Reporting System (NORS) in an effort to increase HuNoV outbreak reporting for the purposes of characterizing differences in mode of transmission. What is the relative importance of the various transmission routes, including foodborne, in the burden of HuNoV disease in the United States?
   - the information gaps for which additional epidemiological or microbiological research or improved public health surveillance might help in controlling foodborne transmission of HuNoVs; and
   - the benefit of a sequence-based strain subtyping method (for example the information collected by the CDC CaliciNet initiative). What additional information could be collected to increase the value of the CaliciNet system? For instance, what would be needed to use CaliciNet to determine whether HuNoV genotypes vary as a function of route of transmission or specific food vehicle(s)?

2) Which types of foods can be attributed to foodborne HuNoV infections? How common is HuNoV contamination in various foods **before** they reach final preparation and point of service (i.e., during production, harvest, and processing)? What are the most likely mechanisms of such contamination?

3) Are there conditions or food matrices for which the current minimum cooking temperatures in Part 3-4 of the FDA 2009 Food Code might be inadequate for destruction or inactivation of HuNoVs? What temperature must molluscan shellfish be heated to in order to inactivate HuNoVs and is this temperature reached when the product is prepared in traditional ways such as steaming for culinary (but not food safety) purposes? Is foodborne transmission of HuNoVs affected by the holding temperature of food? Does recontamination of cooked foods present increased risk for transmission? Considering only preparation and point of service, how likely is food to be contaminated at this phase?

4) What factors most significantly affect the efficacy of removal and/or inactivation of HuNoVs from surfaces and hands when using common cleaning and sanitizing practices? What is known about HuNoV survival, persistence, and transfer on and between foods and surfaces? Are there any concerns about HuNoV survival on

surfaces that have been subject to the minimum cleaning and sanitization requirements specified in 4-6 and 4-7 of the FDA 2009 Food Code?

5) What methods exist for the detection of HuNoVs in foods and environmental samples and what are the limitations of these methods? How should the issue of virus infectivity be approached when using molecular-based assays? What is the potential for a standard method to be developed and used? What is the value of existing and emerging cultivable surrogate viruses for studying methods development, virus persistence and inactivation, sanitation efficacy, etc.?

6) What interventions are available, or might be available in the near future, to reduce or eliminate the likelihood of HuNoV contamination for at-risk foods? Please consider interventions based on:

- Separating ill or infectious persons (including asymptomatic) from foods/time interval for when ill food workers[†] can return to work;
- Reducing contact with contaminated hands;
- Cleaning and sanitizing hands and food contact surfaces;
- Using appropriate procedures in response to incidents of vomiting or diarrhea that occur at food establishments;
- Focusing on interventions upstream from final preparation and point of service.

7) Discuss the possible impact and burden of HuNoV illness if the preventive controls, such as those specified in the Parts 2-2, 2-3, and 3-3 of the FDA 2009 Food Code for retail operations, were also required of producers and processors of ready-to-eat foods.

8) What data are available, and what data are still needed, to conduct a formal quantitative microbial risk assessment of HuNoV transmission in high-risk commodities? Please take into account exposure (levels/frequency) and dose response. When addressing exposure, consider the potential (if any) for zoonotic and secondary transmission of HuNoVs.

9) Do certain types of facilities, such as schools, long-term care facilities, restaurants, cruise ships, airplanes, or carriers of foods in interstate conveyance, require a specific HuNoV control strategy? If this need exists, the Committee should develop a generic plan by which to control HuNoV transmission in restaurants and institutions that could be used as a template upon which facility-specific plans could be based.

1.2 Public Health Focus

[†]For the purposes of this document, the terms “food worker”, “food employee”, “food handler”, “food preparer” and “restaurant employee” should be considered synonymous with the term “Food employee” defined in the 2013 Food Code (US FDA Food Code, 2013) as “an individual working with unpackaged food, food equipment or utensils, or food-contact surfaces”.

The Federal agencies that sponsor and/or participate in NACMCF develop specific charges related to attaining the best **scientific** advice to aid in improving public health around a certain issue---in this case, HuNoV control in foods. The charter specifies NACMCF as a scientific advisory committee to aid regulatory and public health agencies to improve policy, procedures, and actions to address the issue and not to make such policy. However, it is pertinent and useful to the Committee to understand current policy or thinking, any drawbacks or barriers (e.g., legal constraints) to help give context to recommendations by NACMCF as guidelines for future scientific investigations (e.g., investigator initiated, agency program initiated, broader government initiatives such as HHS/NIH or Executive Office of the President/Office of Science and Technology Policy).

1.2.1 FDA's Foods Program and Norovirus:

FDA's Foods Program mission is to promote and protect the public health and economic interest by ensuring that the food and feed supply is microbiologically, chemically, nutritionally, and toxicologically safe and wholesome and cosmetics are safe; and that food and cosmetic products are honestly and accurately labeled. The FDA Foods Program consists principally of activities of the Center for Food Safety and Applied Nutrition (CFSAN) and field programs of the Office of Regulatory Affairs (ORA). The Center for Veterinary Medicine (CVM) has a role in animal feed and veterinary drug safety for animals, including those destined for human consumption. FDA conducts inspections of production, processing, and storage facilities for the food products it regulates. Sampling and testing will occur when violations are cited, in good manufacturing practices (GMPs), sanitation, and, where applicable, deviations from Hazard Analysis Critical Control Points (HACCP) programs. Additionally, CFSAN issues targeted surveillance assignments to ORA for high risk foods, high risk situations (e.g., food service for high profile national events such as political conventions), and certain emergency response situations (e.g., outbreaks) to obtain a short term assessment of pathogen prevalence. FDA's Foods Program is unique relative to FSIS (and FDA's own drug, medical device, and biologics centers) because the predominant focus for ensuring food safety relies mostly on post-market activities which require the documentation of risk. To fully appreciate the significance of this food protection mission, however, it must be understood that the underlying assumption of the laws FDA enforces is that foods are safe. Thus, with the exception of certain pre-market food and feed additive and labeling requirements, FDA must rely on post-market surveillance and scientific evidence to prove that a product is a threat to public health to take action against it.

Regulatory Public Health and Norovirus:

FDA regulatory actions based on norovirus contamination to date are handled on a case-by-case basis. Norovirus in food has typically been classified as a Class 2 type of recall situation in terms of health hazard evaluation (i.e., use of, or exposure to, a violative product may cause temporary or medically reversible adverse health consequences or where the probability of serious adverse health consequences is remote) (US FDA, 2014). However, in this evidentiary building situation, FDA would need to consider the fact pattern of the case, test results, observations, if any, about insanitary conditions, corrective actions, etc. in determining the charge and an appropriate enforcement strategy.

FDA could consider charging adulteration under two sections of the Federal Food Drug & Cosmetic Act (FFDCA) described below if norovirus "was found" in a food sample:

- Section 402(a)(1) because the food bears or contains a poisonous or deleterious substance which may render it injurious to health.

The burden on the agency here would be to show the food 1) contained the virus (i.e., through validated testing), 2) that the virus particles are capable of causing human infection, and 3) that there is a reasonable likelihood of harm to the consumer. Taking this approach would depend significantly on whether the laboratory methods in use could distinguish between detection of viral particles as inert chemical versus infectious particles that are capable of causing human disease. Presently, the validated molecular methods used to detect and estimate levels of norovirus do not distinguish between viable, infectious and non-viable, non-infectious viral particles.

- Section 402(a)(4) because the presence of norovirus in the food demonstrates that it has been "prepared, packed or held under insanitary conditions whereby it may have become contaminated with filth or whereby it may have been rendered injurious to health."

In this posture, the burden on the agency is to establish that the food was 1) "prepared, packed or held" by the firm; 2) conditions of the growing/harvest/processing/packing/holding are insanitary (e.g., usually through observations or other evidence that show a direct route of contamination [e.g., sewage/fecal contamination]), and finally, 3) that there is a reasonable likelihood of harm to the consumer. The finding of norovirus in food would not necessarily get FDA to the insanitary conditions that caused the contamination (unless strong scientific evidence rules out other sources).

There is a third possible regulatory strategy that FDA could consider based on the Public Health Service Act.

- section 361(b) of the Public Health Service Act to prevent the introduction, transmission, or spread of communicable disease.

This approach is considered when food is contaminated but has not entered into interstate commerce. A food must be in interstate commerce for FDA to have authority under the FFDCA.

FDA has no specific norovirus policy other than what is generally applied for other microbiological contaminants. FDA has not had norovirus import alerts or related domestic regulatory actions, other than a 2011 Korean molluscan shellfish situation, described below, and the Class 2 recalls seen from time to time with shellfish (US FDA, 2014. FDA Warns…) However, FDA does have an Import Alert for Hepatitis A Virus in green onions, which demonstrates the scientific evidentiary building steps necessary to take regulatory action within the FFDCA. (US FDA, 2011 Import Alert)

Bivalve Molluscan Shellfish and Norovirus:

Some further insights on the FDA policies and actions related to human noroviruses may be learned from the history related to a particular food commodity(s)--bivalve molluscan shellfish. Regulatory decisions made by FDA during a 2011 Korean oyster illness outbreak serve to illustrate (US FDA, 2012). In this case, there were FDA observations and likely sources of contamination that were identified beyond the testing for norovirus particles alone that supported using the filth (human sewage) part of the 402(a)(4) charge.

The National Shellfish Sanitation Program (US FDA, 2011 NSSP), a cooperative program between FDA and the states covers raw and raw, frozen bivalves, which include all species of oysters, clams, mussels, and whole or roe-on scallops (scallop adductor muscle only is exempt). When shellfish are implicated in a norovirus illness or outbreak, and the source of the shellfish is determined, the implicated harvest area is investigated and closed to further harvest within 24 hours by the State Shellfish Control Authority (the Authority), and voluntary recalls of shellfish in the distribution chain from that area are directed by the Authority. These requirements are intended to protect consumers by putting an end to further illnesses caused by contaminated shellfish from the implicated area.

Noroviruses are often introduced post-production by food handlers. This scenario probably is not the case for molluscan shellfish. When shellfish-associated norovirus illness outbreaks have occurred, the causes determined have always involved contamination of the harvest area waters.

Reconditioning of raw and raw, frozen shellfish implicated in illnesses is not an option permitted under the NSSP. Doing so would thoroughly undermine a founding principle of the NSSP, which is the classification of shellfish areas according to the relative absence or presence (levels) of fecal contamination (sanitation) determined by sanitary surveys and verified by water quality monitoring. Both the U.S. (NSSP) and the EU (CODEX) rely on classification schemes to ensure that shellfish are safe for raw consumption. All commercial shellfish harvested in the U.S. and the FDA-Memorandum of Understanding (MOU)-countries and marketed in the U.S. must meet the NSSP safety standards set for raw molluscan shellfish. In the future when noroviruses derived from sewage are determined as the cause of shellfish-associated illness outbreaks, it is likely that FDA will continue recommending, on a case-by-case basis, recalls covering all market forms of implicated shellfish, whether they are raw, raw frozen, breaded, pickled, or canned.

Retail and Foodservice Establishments and Norovirus:

More than 2,300 state, local and tribal agencies regulate the retail food and foodservice industry in the United States. They are responsible for the inspection and oversight of over 1 million food establishments - restaurants and grocery stores, as well as vending machines, cafeterias, and other outlets in health-care facilities, schools, and correctional facilities.
The Public Service Act [42 USC 243] establishes FDA's authority for providing assistance to state and local governments in the prevention and suppression of communicable diseases. Assistance provided to local, state, and federal governmental bodies is also based on FDA's authorities and responsibilities under the Federal, Food, Drug, and Cosmetic Act [21 USC 301]. FDA strives to promote the application of science-based food safety principles in retail and

foodservice settings to minimize the incidence of foodborne illness. FDA assists regulatory agencies and the industries they regulate by establishing a model for regulation of food establishments, scientifically-based guidance, training, program evaluation, and technical assistance.

The FDA Food Code assists food control jurisdictions at all levels of government by establishing scientifically sound technical and legal basis for regulating the retail segment of the food industry. The FDA Food Code is neither federal law nor federal regulation and is not preemptive. It represents FDA's best advice for a uniform system of regulation to ensure that food at retail is safe and properly protected and presented. Food Code provisions are designed to be consistent with federal food laws and regulations, and are written for ease of legal adoption at all levels of government.

State and local regulations modeled after the Food Code define the preventive controls that operators of food establishments must employ to satisfy the conditions of their permit to operate and to prevent foodborne illness. The Food Code addresses controls for risk factors and establishes key public health interventions to protect consumer health.

The FDA Food Code contains several provisions that address the prevention and control of foodborne pathogens, including Norovirus, in the retail and food service setting. Among other things, the Food Code contains requirements that seek to prevent:

- workers who may be infectious with Norovirus from working with food,
- the contamination of foods by asymptomatic employees;
- the cross-contamination of foods via contaminated surfaces and equipment;
- foods that may be contaminated elsewhere in the supply chain form being offered for sale or service.

In recognition of a common Norovirus transmission pathway, the Food Code was recently revised to require food establishments to establish procedures for employees to follow when responding events that involve the discharge of vomitus or fecal matter into food or onto surfaces in the food establishment.

1.2.2 CDC's Food Safety Public Health Program and Norovirus:

CDC works with state and local health departments to conduct national surveillance for many infections, including those often transmitted through foods. Notifiable diseases are reported to CDC and summarized periodically. Norovirus is not notifiable, nor are sporadic infections tracked in specialized surveillance systems like FoodNet, that depend on clinical laboratory diagnosis. Most outbreaks of illness are detected and investigated by local and state health departments; CDC assists them on request and helps detect dispersed or multistate outbreak. CDC also investigates outbreaks of gastroenteritis on cruise ships arriving to the US from international waters, through the Vessel Sanitation Program (VSP). Four outbreak reporting systems in the U.S. provide information about investigated outbreaks of norovirus infection. Local and state health departments have reported foodborne and waterborne outbreaks to CDC for many years, including those caused by norovirus, via the Foodborne Disease Outbreak Surveillance System (FDOSS) and Waterborne Disease Outbreak Surveillance System (WDOSS)(CDC, 2011 Surveillance Foodborne). In 2009, this outbreak reporting was expanded to include outbreaks of acute gastroenteritis due to person-to-person and animal contact transmission on a common platform called NORS (National Outbreak Reporting System) (CDC, 2011 NORS). Also in 2009, a public health laboratory-based surveillance network was launched

that performs sequence-based subtyping on norovirus identified in outbreaks, called CaliciNet. State public health laboratories test specimens from suspected norovirus outbreaks, and report the genotype of confirmed norovirus outbreaks to CaliciNet, along with limited descriptive information. In addition, outbreaks of AGE on international cruise ships are not reported to NORS, but are tracked separately (CDC, 2014 VSP Outbreak Updates). In 2012, five state health departments began reporting standardized epidemiological (using NORS data) and laboratory data (using CaliciNet) on investigations of HuNoV outbreaks. This program, the Norovirus Sentinel Tracking and Testing (NoroSTAT) network, provides real-time information on HuNoV outbreaks (CDC, 2014 NoroSTAT).

1.2.3 FSIS's Foods Program and Norovirus:

The Food Safety and Inspection Service (FSIS) is the public health agency in the U.S. Department of Agriculture responsible for ensuring that the nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged (USDA, 2014). The FSIS monitors domestic and imported meat, poultry, and processed egg products for bacterial contamination, residues of pesticides, drugs, and other chemicals through implementation of HACCP and verification testing. The FSIS is actively involved in recalls and trace-back or -forward activities for products that may be adulterated and/or related to foodborne outbreaks. For the meat, poultry, and egg products regulated by the FSIS, the pathogens with the greatest impact in the public health are bacterial agents Shiga toxin-producing *Escherichia coli* (such as *E. coli* O157:H7), *Salmonella*, *Campylobacter*, and *Listeria monocytogenes*, while viral agents such as norovirus, and parasitic agents such as *Toxoplasma gondii* are also of concern. The FSIS generally would handle norovirus contamination of a meat, poultry, or processed egg product similarly as described by FDA albeit with a few noted considerations. The adulteration provisions that FSIS would rely upon are contained in three separate statutes: The Egg Products Inspection Act (EPIA), 21 U.S.C. 1033, paragraphs (a)(1) through (a)(8); the Federal Meat Inspection Act (FMIA), 21 U.S.C. 601, paragraphs (m)(1) through (m)(9); and the Poultry Products Inspection Act (PPIA), 21 U.S.C. 453, paragraphs (g)(1) through (g)(7). Under the EPIA, FMIA, and PPIA, product cannot enter commerce unless FSIS has inspected the product, found it to not be adulterated, and applied the mark of inspection to the product. In performing its inspection, FSIS takes into consideration whether the establishment is effectively implementing its food safety system, including its HACCP plan and Sanitation SOPs, and specifically whether the product's intended use is to be not-ready-to-eat (NRTE) or ready-to-eat (RTE). When speaking of NRTE products regulated by FSIS, only meat or poultry products are considered because by statute all processed egg products are required to be designated as RTE at the time the mark of inspection is applied.

Designation of adulteration of RTE meat, poultry, or processed egg products due to an association with norovirus is relatively straight forward. As described above in Section 1.2.1 for FDA, FSIS would rely upon (a)(1), (m)(1), or (g)(1) of the EPIA, FMIA, or PPIA, respectively, if the RTE food contained any infectious norovirus, the food would bear or contain a deleterious substance that may render it injurious to health. By definition, an RTE food is in a form that is edible without additional preparation to achieve food safety. Thus, the infectious norovirus would not be expected to be destroyed through further handling and preparation prior to consumption. All RTE products that contain infectious norovirus would be subject to FSIS

retention (if still under the control of the inspected facility) or detention and seizure (if in commerce). Product released into commerce before a determination of adulteration is made likely would be subject to a Class I recall in that there is a reasonable probability that the use of the product will cause serious, adverse health consequences or death. FSIS generally would not make the determination that NRTE meat or poultry product contaminated with norovirus would be adulterated under (a)(1), (m)(1), or (g)(1) of the EPIA, FMIA, or PPIA, respectively, because of the certainty associated with whether a full lethality treatment typical of meat or poultry (e.g., 71.1°C/160°F for meat or 73.9°C/165°F for poultry) is sufficient to destroy the virus and render it safe to consume. Of note, NACMCF previously identified that the 73.9°C (165°F) temperature for poultry provides a margin of safety against *Salmonella* and less heat-resistant pathogens such as avian influenza type A (H5N1) and *Campylobacter* (NACMCF, 2007).

For both NRTE and RTE meat or poultry products, or for RTE processed egg products, FSIS likely also would make a similar determination as FDA in that the product is adulterated under statutory sections (a)(4), (m)(4), or (g)(4) of the EPIA, FMIA, or the PPIA, respectively, whereby the product is found to have been prepared, packed, or held under insanitary conditions whereby it may have been rendered injurious to health. FSIS uses this provision particularly when sanitary conditions are in question during the production or handling of the product before or after FSIS inspection and the product is associated with illness but the infectious agent hasn't been identified in a specific product lot of food. In addition, unlike FDA statutes, FSIS may find that for both NRTE and RTE meat or poultry products or for RTE processed egg products, FSIS may determine that product is adulterated under (a)(3), (m)(3), or (g)(3) of the EPIA, FMIA, or the PPIA, respectively, whereby the product is unsound, *unhealthful*, unwholesome, or otherwise unfit for human food. FSIS uses this statute particularly when product is associated with illness, the infectious agent hasn't been identified in a specific product lot of food, and the production or handling of the product doesn't indicate insanitary conditions were a likely contributing factor. FSIS recently clarified its current thinking regarding adulteration determinations for NRTE meat or poultry products when associated with an outbreak, citing (m)(3) and (m)(4) for the FMIA and (g)(3) and (g)(4) for the PPIA (USDA FSIS, 2012). FSIS does not have authority to cite the Public Health Service Act, unlike the FDA, which does.

1.2.4 USDA Food and Nutrition Service (FNS) Office of Food Program and Norovirus

The USDA Food and Nutrition Service (FNS) Office of Food Safety provides child nutrition professionals with food safety resources to promote the safety of foods served through Federally funded nutrition assistance programs, such as the National School Lunch Program (NSLP). Based on analyses of CDC data from NORS and its predecessor Electronic Foodborne Outbreak Reporting System (eFORS), the FNS Office of Food Safety has identified HuNoV as the leading cause of reported foodborne illnesses and outbreaks in schools (Venuto, 2014 in press). School settings are closed environments with unique risk factors for virus transmission such as close contact among children, the use of common restroom facilities, exposure to common toys and other fomites, and the sharing of foods.

Through a partnership with the National Food Service Management Institute (NFSMI) at the University of Mississippi, the FNS Office of Food Safety provides resources on norovirus prevention to school and child nutrition professionals. These resources include in-person and

online courses, videos, standard operating procedure (SOP) templates, posters, and fact sheets (www.nfsmi.org/norovirus). In addition, the FNS Office of Food Safety collaborated with the National Education Association (NEA) Health Information Network to produce the informational booklet on norovirus prevention entitled, The Stomach Bug Book: What School Employees Need to Know. (NEA, 2012)

1.2.5 NOAA and NMFS's Food Safety Programs and Responsibilities

The National Oceanic and Atmospheric Administration (NOAA), National Marine Fisheries Service is the federal agency, a division of the Department of Commerce, responsible for the stewardship of the nation's living marine resources and their habitat. NOAA's National Marine Fisheries Service is responsible for the management, conservation and protection of living marine resources within the United States' Exclusive Economic Zone (water three to 200 mile offshore). NOAA's National Marine Fisheries Service assesses and predicts the status of fish stocks, ensures compliance with fisheries regulations, and works to reduce wasteful fishing practices. Under the Marine Mammal Protection Act and the Endangered Species Act, NOAA's National Marine Fisheries Service recovers protected marine species (i.e. whales, turtles) without unnecessarily impeding economic and recreational opportunities. Through its Seafood Inspection Program (SIP) and National Seafood Inspection Laboratory (NSIL), NOAA plays an important role in food safety. The SIP and NSIL respond to seafood safety and aquatic animal health issues and episodic events and has the capability to respond quickly to environmental disasters. The SIP is a voluntary, fee-for-service program for inspection and certification of fishery products for quality and safety. The mission of SIP involves providing assistance to industry and consumers in improving the overall quality and marketability of seafood and ensuring that all processing firms are compliant with the FDA and Department of Commerce regulations. The SIP supports the FDA's mission by enforcing regulatory requirements and referring non-compliant seafood and processing firms to the FDA. A variety of services, including in-plant inspections, product evaluation and grading, HACCP services, and consultation for regulatory compliance, are offered to the industry. The NSIL serves as the official government reference laboratory and provides scientific support to the SIP.

1.3. Committee's Approach to Answering the Charge

After review of the charge to the Subcommittee, reference materials, and presentations by invited subject matter experts, the subcommittee discussed options for approaching the charge. It was decided to establish working groups to address related questions that would topically fit under a document Chapter or section. The charge questions are indicated in the document as *italicized text* at the outset of each Chapter. In developing any document of this deliberative nature, there are areas that cross-over or cross-reference several Chapters. Where clear and applicable, the charge questions relevant to certain parts of the document other than the intended Chapter are indicated in parenthetical statements. Therefore, the approach by the Committee carries both a structured question-by-question approach and a comprehensive approach to establish relationships and points of synergy.

Chapter 2: THE EPIDEMIOLOGY OF NOROVIRUS INFECTIONS (QUESTIONS 1 AND 2)

Charge 1: What is known about the incidence and public health burden of HuNoV infection in the United States and in other developed countries? Are there population subgroups at increased risk of more severe disease? Please consider in your response:

- ***the value of improvements in compliance with the CDC National Outbreak Reporting System (NORS) in an effort to increase HuNoV outbreak reporting for the purposes of characterizing differences in mode of transmission. What is the relative importance of the various transmission routes, including foodborne, in the burden of HuNoV disease in the United States?***
- ***the information gaps for which additional epidemiological or microbiological research or improved public health surveillance might help in controlling foodborne transmission of HuNoVs; and***
- ***the benefit of a sequence-based strain subtyping method (for example the information collected by the CDC CaliciNet initiative). What additional information could be collected to increase the value of the CaliciNet system? For instance, what would be needed to use CaliciNet to determine whether HuNoV genotypes vary as a function of route of transmission or specific food vehicle(s)?***

Incidence and Public Health Burden in the US and Other Developed Countries

In sporadic cases of gastroenteritis in the community, norovirus infection is often suspected on clinical grounds but specimens are rarely collected and submitted for laboratory testing. Simple diagnostic test development has been hindered by the inability to cultivate these viruses, and by challenges in developing sensitive assays. Two commercial diagnostic kits for norovirus have been cleared by the FDA, but diagnostic applications are limited to outbreak settings. Even estimates based on careful laboratory studies may underestimate the true risk of disease, because of limited test sensitivity. Therefore, public health surveillance for individual cases of norovirus infection has only recently become possible, and current estimates of the incidence of infection depend primarily on extrapolation from the results of specialized research studies. Recently, it has been estimated that noroviruses cause approximately 16% of all AGE in the U.S. (Hall, Lopman et al., 2013). In a comprehensive estimate of the health burden of foodborne infections, norovirus caused the most cases annually of domestically-acquired foodborne illness (5,500,000 annually), ranked second in hospitalizations (15,000) and fourth in deaths (150) (Scallan et al., 2011).

Estimating the frequency of infection

Estimates of the frequency of norovirus infection have used three main approaches. One approach is to conduct a prospective study in a population cohort, in which aggressive efforts are made to obtain diagnostic specimens from all cases of AGE to determine the etiology of the illness. Such studies require substantial resources, are rarely conducted for more than a year, and only a small number have been done. A second approach based on the etiologic fraction of AGE illness is to estimate the frequency of AGE in a population, and then estimate the fraction of that AGE that is due to norovirus. A third approach is to estimate the contribution of norovirus indirectly from analytic models of seasonal variation in hospitalizations or other diagnosis-specific measures. These indirect estimates can usefully complement other measures. Estimates of the frequency of norovirus infection vary, depending on methodologies used and on country of study. Earlier studies based on less sensitive diagnostic methods may have

underdiagnosed norovirus infections substantially, compared to more recent studies based on RT-PCR diagnosis. As RT-PCR is more often used, the burden of norovirus can be more accurately measured.

Estimates of incidence of AGE due to noroviruses (See Table 1 – Estimates of the general population incidence of norovirus disease in the United States and other countries (adapted from Hall, Lopman et al, 2013).

Incidence in the United States

Since 1999, three estimates of the annual incidence of norovirus illness in the general population have been published (Table 1**– Estimates of the general population incidence of norovirus disease in the United States and other countries (adapted from Hall, Lopman et al., 2013)**. The earliest estimate was 23,000,000 cases, or 8.6 cases per 100 based on population surveys of the incidence of AGE in the U.S., and an estimate that 11% was due to noroviruses based on data from the Netherlands (Mead et al., 1999). A revised estimate of 21,000,000 or 7.0 cases per 100 population was based on updated survey data, and an etiologic fraction of 11% again derived from studies in other countries (Scallan et al., 2011). The two estimates are of similar magnitude, though differences in methods and input data hinder direct comparison.

The most recent estimate was based on a prospective cohort study of patients presenting with AGE to a health maintenance organization in Georgia, testing specimens with RT-PCR analysis (Hall, Rosenthal et al., 2011). The community incidence of norovirus AGE was estimated to be 6.5 per 100 persons, (with 90% confidence interval (CI) 3.7 – 12), or 16% of all AGE, equivalent to 19 million illnesses per year in the U.S. Thus, recent estimates for illness cluster around 6.5 – 7.0 illnesses per 100 persons per years, or approximately five illnesses per person over an average life expectancy of 79 years (Hall, Lopman et al., 2013).

The frequency of outpatient visits for all age groups was estimated using a time series regression model applied to an insurance claim database (Gastañaduy et al., 2013). The model estimated that 13% of all AGE-associated outpatient visits are due to norovirus, at an annual rate of 57 per 10,000 persons, or 1.7 million visits annually.

The number of children less than five years of age brought to medical attention with norovirus AGE was recently estimated (Payne et al., 2013). The annual incidence of outpatient visits for norovirus infection was 319 per 10,000 children less than five years, and that of emergency department visits was 141 per 10,000. Applied to the national population, this rate means 908,000 outpatient and emergency department visits annually for this age group.

Incidence in other developed countries

Studies in three other countries have estimated the incidence of norovirus AGE in the general population. In the Netherlands, a prospective population-based cohort study estimated incidence of gastroenteritis and the associated pathogens in 1999 (de Wit et al., 2001). In this study, 11% of AGE was attributed to norovirus, giving a population incidence of 3.1 per 100 persons. This Dutch estimate was updated to 3.8 per 100 persons for the year 2009, using more recent data

(Verhoef et al., 2013). In England and Wales, two large population–based studies measured the incidence and etiology of enteric infections in the community and among cases that presented to general practitioners (GPs)(Wheeler et al., 1999; Tam et al., 2012). The first study, (using data collected in 1993-1996) after analyzing specimens with improved diagnostic methods and incorporating a measure of viral load, found a final adjusted incidence of norovirus infection in the community of 4.5 per 100 population (95% CI 3.8 – 5.2) (Phillips et al., 2010). The second study (in 2007-2009), using comparable improved diagnostic methods, estimated that 17% of AGE episodes were attributed to norovirus and that the norovirus AGE incidence in the community was 4.7 per 100, where norovirus AGE leading to a visit to a GP was 2.1 per 100 (Tam et al., 2012). In Canada, an approach similar to that of the modeled US estimates yielded an estimated 2006 incidence of 10.4 per 100 persons (Thomas et al., 2013). To estimate the global prevalence of norovirus infection in cases of AGE, a WHO work group reviewed 175 published studies (Ahmed et al., 2014). This meta-analysis found that the pooled prevalence was 18% (95% CI 17 – 20%). Studies included were based on PCR diagnosis, lasted at least a year, and were published between 2008 and 2014.

Incidence of hospitalization due to norovirus in the United States (See Figure 1 - Estimates of the annual burden and individual lifetime risks for norovirus disease in the United States. (Adapted from Fig 3, Hall, Lopman et al., 2013).

Two recent estimates suggest that 56,000 to 71,000 norovirus hospitalizations occur each year in the U. S. In 2011, 56,000 hospitalizations from norovirus infections were estimated to occur each year, 0.03% of all norovirus infections, or 19 hospitalizations per million persons (Scallan et al., 2011). This figure was derived by applying the etiologic fraction for norovirus to the estimated total number of AGE hospitalizations. Using a second method, time-series regression models applied to hospitalization data over an 11-year period, norovirus was estimated to have caused 7% of AGE hospital discharges, giving an annual mean of 71,000 (Lopman et al., 2011).

Incidence of death due to norovirus in the United States (See Figure 1– Estimates of the annual burden and individual lifetime risks for norovirus disease in the United States. (Adapted from Fig 3, Hall, Lopman et al., 2013).

Two recent estimates of the number of norovirus-associated deaths range from about 570 to 800 per year in the U.S. In one, 571 deaths from norovirus infection were estimated to occur each year, or 1.7 per million persons, by applying the same etiological fraction to the total estimated number of deaths due to AGE (Scallan et al., 2011). In a second, 797 deaths were estimated to occur, or 2.7 per million persons, using a time series regression model applied to reported AGE death data (Hall, Curns et al., 2012).

Frequency, size and location of norovirus outbreaks

<u>Outbreaks in the United States</u>

<u>Frequency of outbreaks</u>. Approximately 1000 norovirus outbreaks are reported each year in the U.S., a quarter of which are foodborne. Norovirus accounts for about half the foodborne outbreaks of known etiology. Suspected foodborne outbreaks may be more likely to be

investigated and reported than person-to-person outbreaks, though the distinction between foodborne and person-to-person transmission is not always clear.

Between 2001 and 2008, 2,922 confirmed or suspected norovirus outbreaks were reported to the Foodborne Disease Outbreak Surveillance System (FDOSS), a mean of 365 outbreaks each year, along with 10,324 associated cases, 156 hospitalizations, and 1 death each year (Hall, Eisenbart et al., 2012). From 2009 through 2012 a combined total of 4,318 outbreaks were reported to the expanded NORS for which norovirus was confirmed or suspected (Hall et al., 2014). This is an annual incidence of 3.5 reported norovirus outbreaks per million people. Among these, 252 foodborne outbreaks per year were foodborne, representing 48% of all reported foodborne outbreaks of known etiology. The decline in the annual number of reported foodborne norovirus outbreaks from 365 in 2001-2008, to 252 in 2009-2012 may indicate that some outbreaks which would previously have been reported as foodborne may be reported as person-to-person now that NORS provides that option (Gould et al., 2013).

In a four year span from 2009 to late 2013, 3,960 outbreaks were reported to CaliciNet with viral sequence information, along with limited descriptive information (Vega et al, 2014). These outbreaks are not all reported to NORS as well, and are not yet easily linked to the NORS reports. It is likely that the actual number of outbreaks investigated is higher than the numbers reported by either system alone. Finally, a mean of 10 norovirus outbreaks per year occur on international cruise ships as reported to CDC VSP (CDC, 2014 VSP Outbreak Updates).

<u>Size of outbreaks</u>. The size of reported norovirus outbreaks varies by transmission route and setting. Among outbreaks reported to NORS in 2009-2010, the mean size was 36 illnesses per outbreak. Among person-to-person outbreaks, the mean size was 44, and the median was 33 (Wikswo and Hall, 2012). Among 2,922 foodborne norovirus outbreaks reported from 2001-2008, the mean size was 28 cases, and the median was 14 (Hall, Eisenbart et al., 2012). This varied by location according to where the food was prepared; the median was 43 cases for schools, 35 for nursing homes, 16 for private homes or events, and 12 for restaurant- or deli-associated outbreaks.

<u>Location of outbreaks</u>. Most norovirus outbreaks are recognized in commercial food service settings, while most person-to-person outbreaks are recognized in nursing homes, as well as in other closed environments and institutional settings such as hospitals, schools, and cruise ships. Among the 2,922 foodborne norovirus outbreaks reported between 2009 and 2012, with known setting, 81% (725 outbreaks) occurred in commercial food service settings, 4% (37) in private settings, 2% (28) in institutional settings and 13% (114) in other settings (Hall et al., 2014). Among the institutional settings only 1% (12 outbreaks) were long-term care facilities (Hall et al., 2014). The location of outbreaks is quite different for person-to-person norovirus outbreaks. Among 2,590 non-foodborne outbreaks for which location was reported in 2009-2012, 80% (2,060 outbreaks) were in long-term care facilities, 4% (115 outbreaks) were in hospitals, 6% (148) occurred in schools and 0.1% (32) in private settings. Only 1% (38) occurred in restaurant settings (Hall et al., 2014). Based on CaliciNet data, outbreaks on cruise ships caused 2% of all reported laboratory confirmed norovirus outbreaks between 2009-2013 (Vega et al., 2014).

Seasonality. From the time it was first recognized, norovirus infections have been associated with winter months. Foodborne and person-to-person outbreaks are more common in the winter, but this seasonal concentration is much more pronounced for person-to-person outbreaks. Although norovirus outbreaks occur throughout the year, peak activity occurs between October through April (Figure 2 – Number of reported outbreaks of norovirus infection by month and mode of transmission, 2009-2012, United States (Hall et al., 2014). A similar pattern has been observed in many countries with a temperate climate (Ahmed et al., 2013). Between 2009-2012, 39% of foodborne norovirus outbreaks occurred in the three-month period December through February, while 60% of the non-foodborne outbreaks occurred in this time period (Hall 2014 MMWR). This suggests that the underlying events that leads to foodborne outbreaks may differ somewhat from those that lead to person-to-person outbreaks. An exception is shellfish-associated outbreaks, which are strongly seasonal; 77% of shellfish-associated norovirus outbreaks reported to CDC 1973-2006 occurred between October and March (Iwamoto et al., 2010).

Genotype variation. Norovirus genotype distribution varies in secular trends (consistent trend over time), seasonality and transmission route. The diversity of genotypes complicates generalizations and the frequent emergence of new GII.4 variants complicates predictions. Approximately 40 norovirus genotypes within six genogroups (GI through GVI) have been described, of which GI, GII, and GIV cause human illness (Green, 2013). The majority (~90%) of norovirus outbreaks worldwide are caused by GII. The application of sequence-based subtyping as part of norovirus surveillance has demonstrated shifts in genotype over time. In the U. S., the proportion of GII.4 viruses increased from 5% of outbreaks characterized in 1994 to 85% in 2006, an increase punctuated by peaks in specific GII.4 variants (Zheng et al., 2010). Between 2009 and 2011, the GII.4 variant New Orleans 2009 predominated in the U.S., while in 2012, the GII.4 variant Sydney 2012 did (Vega et al., 2014). The specific genotypes may also vary in seasonality and transmission route. For example, GII.4 predominates in person-to-person outbreaks, most of which occur during the winter season, while other GII viruses as well as certain GI viruses are more often associated with foodborne outbreaks and are less often associated with the winter season (Table 2 -Epidemiological characteristics of outbreaks of norovirus infection caused by genotype GII.4 strains and nonGII.4 strains (Desai et al., 2012; Leshem, Wikswo et al. 2013; Leshem, Barclay et al. 2013; Matthews et al., 2012; Vega et al., 2014). As with the difference in transmission route by season, these seasonal differences in predominant genotype suggest that the local community transmission leading to person-to-person outbreaks in nursing homes, for example, may have a different underlying epidemiology than those leading to foodborne outbreaks in restaurants.

Outbreaks in other developed nations. Norovirus outbreaks are common in industrialized countries and have similar transmission routes, settings, seasonality, and genogroup and genotype distribution. However, the summary information available about these outbreaks varies considerably. In England and Wales, 1,877 outbreaks of norovirus infection were reported between 1992 and 2000, an average of 209 per year or approximately 4 outbreaks per million persons each year (Lopman et al., 2003). Of these, 85% were person-to-person and 10% were foodborne, of which half also had secondary person-to-person spread. Among the person-to-person outbreaks, 86% were in hospitals or residential facilities, while among foodborne

outbreaks 61% were at food outlets or hotels. Outbreaks in hospitals or residential facilities were strongly seasonal, while outbreaks in other settings exhibited no seasonal variation at all.

A European research network in 13 countries gathered reports of 7,636 outbreaks between 2001 and 2006, reaching an annual incidence in 2005-2006 of 8.2 outbreaks per million persons (Kroneman et al., 2008). Among those outbreaks with reported route of transmission, 88% were person-to-person, 10% were foodborne, and 2% were waterborne. Of those with reported setting, 72% were in health-care institutions (hospitals and nursing homes) while 8% were in restaurants. Genotype II.4 was more likely to be person-to-person than were other genotypes, and more likely to cause outbreaks in health-care institutions.

In Australia, surveillance for outbreaks of AGE in long-term care facilities from 2002 to 2008 found 1,136 outbreaks due to norovirus (Kirk et al., 2010). Norovirus was responsible for 94% of such outbreaks of known etiology and 96% of the outbreak-associated cases. With a case-fatality ratio of 0.3%, norovirus accounted for the largest number of outbreak-associated deaths associated with any pathogen in that setting. Only one of the norovirus outbreaks in long-term care facilities was foodborne.

In Japan, norovirus is the most frequent cause of foodborne outbreaks. In nine seasons from 2002 to 2011, 2,890 outbreaks were reported or 321 per year (incidence 2.5 outbreaks per million persons) with a mean of 41.5 cases per outbreak (National Institute Infect. Dis., 2011). Of these, 64% were related to restaurants.

Population groups at higher risk for severe norovirus infections

A number of variables affect susceptibility of an individual to noroviruses, including age, genotype of infecting strain, the route of transmission, immune-competence of infected individuals, and immunity (see also section on Infectious Dose and Immunity below). Age is a primary variable in the likelihood that an individual will develop acute gastroenteritis and seek outpatient treatment, visit an emergency room, be hospitalized, or die from the infection (See Figure 3 - Incidence of outpatient visits, emergency department visits and hospitalizations due to norovirus infection by age, United States. (Adapted from Hall, Lopman et al., 2013). Children under five years have the highest burden of disease (Hall, Lopman et al., 2013), and are most likely to be seen in an outpatient setting or emergency room. They are also the group most likely to be hospitalized. Among children less than five years, hospitalization for norovirus-associated AGE was 7.2 per 10,000 in 2009-2010, or 2.2% of the norovirus-associated outpatient visits (Payne et al., 2013). Applied to the national population, this rate represents 14,000 hospitalizations annually. Rates of hospitalization were highest in the first two years of life. Persons 65 and over are the group most likely to die from infection. The effect of advanced age means that the case fatality ratio (CFR) in health care settings (primarily nursing homes) is 396 per million cases, compared to only 2/million cases in community settings (Desai et al., 2012). Similarly, CFRs are highest for outbreaks due to person-to-person spread compared to those due to food or water transmission. Death rates also depended on the infecting strain, as death rates in outbreaks in health care settings were 6-fold higher if caused by genotype GII.4 strains, compared to those caused by non-GII.4 strains (Desai et al., 2012; Table 2 Epidemiological characteristics of outbreaks of norovirus infection caused by genotype GII.4 strains and nonGII.4

strains (Desai et al., 2012; Leshem, Wikswo et al., 2013; Leshem, Barclay et al., 2013; Matthews et al., 2012; Vega et al., 2014).

Immunocompromised patients are particularly vulnerable to norovirus infection and disease (Bok and Green, 2012). Indeed, the incidence of norovirus gastroenteritis in immunocompromised individuals, such as renal and other transplant patients, is estimated to be 17 to 18%. In addition, immunocompromised individuals who are infected with norovirus may shed the virus in their stools for many weeks or even years after acute onset of illness, compared to 20-40 days in immunocompetent hosts. The virus that is shed also differs, as markedly diverse variants are found in the stools of an immunocompromised person, but only a small number of stable viral variants are found in the feces of a normal host. Complications of norovirus disease are greater for immunocompromised patients compared to immunocompetent persons; they can become dehydrated, malnourished and can develop dysfunction of the intestinal barrier. Thus, immunocompromised individuals along with young children and the elderly would make prime candidates for a non-replicating norovirus vaccine.

Infectious dose and immunity

The infectious dose for norovirus AGE is estimated to be very low, while the amount of virus shed in diarrheal feces is high. The dose required to cause infection in 50% of people exposed (ID_{50}) has been estimated to be 18 to 1,000 virions (Teunis et al., 2008). This dose was determined by quantitative real-time reverse transcriptase PCR (qRT-PCR). This is the first estimate using quantitative data on norovirus infectivity as assessed with RNA genome copy number. A more recent study reports estimates that are higher (ID_{50} ranged from 1,320 to 2,800) for a secretor positive test group (Atmar et al., 2013). This more recent estimate is based on human challenge studies using fecal suspensions derived from stool specimens from the original 1968 Norwalk virus that had been passaged through human volunteers (Dolin et al., 1972).

If one applies the estimated low ID_{50} of 18 viral particles together with the reported median peak amount of 9.5×10^{10} viral RNA copies shed per gram of feces from infected volunteers (Atmar et al., 2008), then up to five billion infectious doses of virus might be present in a single gram of feces during peak illness (CDC, 2011 Updated NoV Outbreak Mgmt Guidelines). The combination of infectivity at a low dose and high fecal viral load would account for the high frequency of secondary transmission of the virus. Norovirus was detected in half of the ill volunteers who vomited. The viral shedding in vomitus was 4.1×10^4 per ml of vomitus, much lower than in feces (Atmar et al., 2013).

In volunteer studies, some exposed subjects remain uninfected even after challenge with high doses of the virus (Lindesmith et al., 2003). Some of these appear to be innately resistant to infection with the challenge virus. About 20% of the Caucasian population does not secrete histocompatibility–blood group antigens in saliva, nor express them on the surface of intestinal epithelial cells. These “non-secretors” are resistant to norovirus challenge, which suggests that noroviruses use HBGA antigens as binding ligands. Others did not get sick from the challenge virus due to immunity acquired after a preceding infection. Early human rechallenge studies showed that immunity lasts 6-24 months; however, recent modeling suggests it may last as long as 4-8 years (Simmons et al., 2013). Asymptomatic carriage is documented in human challenge

studies and prospective cohort studies, though the public health implications are uncertain (Simmons et al., 2013; Ahmed et al., 2014).

Charge 2: Which types of foods can be attributed to foodborne HuNoV infections? How common is HuNoV contamination in various foods before they reach final preparation and point of service (i.e., during production, harvest, and processing)? What are the most likely mechanisms of such contamination?

Estimating the fraction spread through food and other modes of transmission.

Norovirus can spread directly from one person to another via contact, or indirectly via contaminated food, water, and fomites, and through airborne droplets of vomitus. The fraction of illness attributable to each of the main modes of transmission has been estimated in several ways. These estimates indicate that most transmission is person-to-person, and that a smaller but important fraction occurs through contaminated food. One way to estimate the fraction of illnesses attributable to each mode of transmission is to collect a series of outbreak investigations. Foodborne outbreaks typically are 10 – 26% of the total number of norovirus outbreaks identified.

The fraction attributable to food has been estimated using outbreak data, though these estimates may reflect a greater tendency to investigate and report foodborne outbreaks than person-to-person outbreaks. In 2011, 26% of all norovirus infections in the US were estimated to be foodborne, based on six years of data on norovirus outbreaks from six states that tracked such outbreaks across multiple transmission modes (Scallan et al., 2011). Following the emergence of genotype GII.4 Den Haag variant in 2006, 79% of outbreaks reported from January 2007 to April 2010 were attributed to person-to-person transmission, leaving only 21% for foodborne and other modes of transmission (Yen et al., 2011). Among norovirus outbreaks reported to NORS in 2009-2012, 69% were person-to-person, 23% were foodborne, 0.4% were environmental, and 0.3% were waterborne; mode of transmission was undetermined for 7% (Hall et al. 2014). In other recently collected data, among 3,060 norovirus outbreaks with routes of transmission reported to CaliciNet between 2009 and 2013, 84% were person-to-person, and 16% were foodborne (Vega et al., 2014). Reports to NORS represent fully investigated outbreaks, but may be biased to include more foodborne outbreaks compared to outbreaks reported through CaliciNet. Outbreaks due to genotype GII.4 were more likely to be associated with person-to-person transmission, while those due to genotypes GI.3, GI.6, GI.7, GII.3, GII.6, and GII.12 were more likely to be foodborne (Vega et al., 2014).

Among norovirus outbreaks reported in England and Wales in the 1990's, 10% were foodborne (Lopman et al., 2003). A similar series in Australia yielded an estimate of 25% (Hall et al., 2005). In a series of outbreaks reported between 2001 and 2006 from 13 European countries, 88% were suspected to be person-to-person, 10% to be foodborne, and 2% waterborne (Kroneman et al., 2008). By comparing the genotypes of norovirus strains found in outbreaks, human feces, and in oysters, a European consortium estimated that 21% of the outbreaks were foodborne (Verhoef et al., 2010).

Estimates have also been generated by "expert elicitation", i.e. systematically consulting a panel of experts. Estimates in the Netherlands, in Canada, and in New Zealand range from 17 – 39% (Havelaar et al., 2008; Ravel et al., 2010; Lake et al., 2010). These estimates are likely to synthesize experience reported directly in outbreaks.

Finally, a case-control study in the Netherlands highlighted the challenge of using this method to attribute norovirus illness by transmission route (de Wit et al., 2003). It was estimated that 16% of cases were related to contaminated foods brought into the home, and up to 60% was associated with food contaminated in the home by an ill person with poor personal hygiene, though this was difficult to separate from the simple presence of the ill family member in the home (de Wit et al., 2003).

Secondary spread and mixed modes of transmission

Transmission through food and via direct person-to-person spread have different dynamics, reflected in differences in seasonality and genotype distribution; the two types of outbreaks have even been described as having two distinct epidemiologic patterns (Lopman et al., 2003). However, these "two epidemiologies" can overlap. It is not unusual for foodborne outbreaks to be followed by some secondary person-to-person transmission, and a person-to person outbreak could lead to a secondary foodborne outbreak if one of the cases happened to be in a foodhandler. Secondary person-to-person transmission was noted for half of the foodborne outbreaks reported in England and Wales in 1992-2000 (Lopman et al., 2003). The extent of secondary transmission has sometimes been documented. In 2009, an outbreak of norovirus infection caused by a previously rare genotype, GII.12, was linked to Gulf Coast oysters served at a restaurant (Alfano-Sobsey et al., 2012). Twenty percent of the affected households had a secondary illness among members who had not eaten the oysters; the authors calculated that there was one secondary case for every five persons made ill by the oysters. The challenge of estimating the fraction of norovirus due specifically to food reflects the sometimes subjective distinction made by investigators between foodborne and person-to-person transmission routes. Secondary transmission, whether foodborne or person-to-person, may be useful to include in models of prevention effectiveness, and effective prevention and control may ultimately require considering norovirus transmission across multiple routes.

Could norovirus be a zoonotic pathogen?

Noroviruses are adapted to many animal hosts, with strong species specificity; inter-species transmission is rarely documented (Bank-Wolf et al., 2010). Most animal noroviruses belong to genogroup III (bovine), genogroup IV (canine), genogroup V (mice), and genogroups VI and VII (canine), genogroups not found in humans. While genogroup II genotypes have been found in swine (GII.11, GII.18, GII.19), these genotypes have not been detected in people (Wang et al., 2005). There is no epidemiological evidence to date that noroviruses adapted to non-human reservoirs cause human illness. There is also little indication that HuNoV can persist in animal hosts. However, several studies suggest that zoonotic transmission is a possibility. For example, gnotobiotic pigs have been experimentally infected with a GII.4 HuNoV strain with evidence of sustained replication in some animals and successful serial passage to other animals (Cheetham et al., 2006). GII.4 and GII.12 RNA has been detected transiently in fecal specimens from pet

dogs in the setting of human family outbreaks (Summa et al., 2012), though detection of the viral genome does not prove the presence of replicating or infectious virions. Norovirus sequences closely related to HuNoV GII.4 viruses have been detected in swine and cattle, and once in pork meat (Mattison et al., 2007). Finally, a recent study showed that humans have antibodies against canine GVI norovirus (Mesquita et al., 2013). These findings suggest that transmission through food or contact with land animals is at least theoretically possible, and that the introduction of a new strain into humans from an animal reservoir cannot be excluded.

Defining the proportion related to specific foods

Outbreak investigations that identify a specific food vehicle provide the most direct information on the specific foods or commodities that transmit norovirus. Reported series of outbreaks provide data with which to allocate the fraction due to each food type. Foods can be categorized in commodity groups, and implicated food vehicles can be simple (containing a single food commodity group) or complex (containing multiple food commodity groups) (Painter et al., 2009). The simple foods most often implicated as vehicles of norovirus foodborne disease are leafy greens, fruits and nuts, and molluscan shellfish (in particular raw or lightly cooked varieties). However, it must be noted that complex food account for the vast majority of norovirus outbreaks.

Among the different foods categories, salads and/or produce account for approximately half of norovirus foodborne outbreaks. Similarly, among all foodborne outbreaks due to leafy greens, norovirus accounts for 36% of illnesses. In an analysis based on foodborne norovirus outbreaks reported from 1998 to 2000 in the U.S., the foods implicated in 76 norovirus outbreaks were salads (26% of outbreaks), produce (17%), sandwiches (13%), meat dishes (11%), baked goods (7%), fish dishes (5%), oysters (3%), and other (18%), (Widdowson et al., 2005). In a more recent series, among 364 foodborne norovirus outbreaks reported to the CDC from 2001-2008 in which simple food categories were implicated as the vehicle, the foods implicated were leafy green vegetables (33% of outbreaks), fruits or nuts (16%), and mollusks (13%), foods generally eaten raw or lightly cooked (Hall, Eisenbart et al., 2012). In 2013, a comprehensive analysis was published of foodborne outbreaks reported from 1998 – 2008 (Painter et al., 2013). Data from outbreaks attributed to both simple and complex food vehicles were used to allocate the fraction of illness to food groups, based on numbers of illnesses, rather than numbers of outbreaks. See Figure 4 - Attribution of norovirus illnesses to specific food commodities based on outbreaks reported 1998-2008, in which simple and complex foods were implicated (Adapted from Painter et al., 2013, Online Table 4). This analysis attributed 36% of norovirus illness to leafy greens, fruits and nuts (15%), dairy products (12%), eggs (7%), vegetables grown on vines or stalks (6%), root vegetables (5%), and mollusks (2%).

In England and Wales, one or more specific food vehicles were reported in 72 of the 184 foodborne norovirus outbreaks reported between 1992 and 2000 (Lopman 03). Among these were oysters (23% of outbreaks), salads and vegetables (20%), poultry (10%), fish (7%), meat (6%), and 34% to other foods. In Japan between 2002 and 2011, among 561 foodborne norovirus outbreaks for which a food type was reported, the implicated food was oysters (62% of outbreaks), sashimi or sushi (21%), confectionery (6%), other shellfish (4%), salads (4%), and

sandwiches (3%) (National Institute Infect. Dis., 2011). Some international variation may be due to a difference in consumption patterns and levels of contamination in these countries.

Using expert elicitation, a panel of 42 experts, using somewhat different food categorizations, estimated that 37% of foodborne norovirus illness in the US could be attributed to produce, 34% to seafood, 9% to luncheon and other meats, 6% to breads and other bakery products, and 4% to beverages (Hoffman et al., 2007). A panel of 21 experts in Canada used a similar instrument to estimate that 30% of norovirus infections in Canada could be attributed to produce, 34% to seafood, 9% to luncheon meats, 4% to breads and other bakery items, and 1% to beverages (Davidson et al., 2011). In the Netherlands, a panel of five experts estimated that 51% of foodborne transmission of norovirus was due to contamination by infected humans in the kitchen. Of the remainder, 32% was due to contaminated seafood, 14% to produce, 10% to grains, and 6% each to beef, pork and poultry (Havelaar et al., 2008).

Points where norovirus contamination of foods occurs

Any food directly exposed to humans shedding the virus or indirectly exposed to fecally contaminated waters, ice or surfaces could transmit the infection if there is no intervening kill step, such as cooking. In 191 foodborne outbreaks in the U.S. from 2001 to 2008 with information on point of contamination, the likely point of contamination for 85% of the outbreaks was during preparation or service and for 15% of the outbreaks it was during production or processing (Hall, Eisenbart et al., 2012). Typical scenarios of contamination include salads and sandwiches contaminated by infected food preparers in the kitchen, fruit or leafy greens exposed to contaminated irrigation waters, and oysters harvested from sewage-contaminated growing waters and eaten raw.

Ill or infected food handlers are often identified in outbreak investigations, indicating that contamination likely occurred in final preparation or service. For example, in foodborne outbreaks reported in England and Wales 1992-2000, infected food handlers were identified in 32 % of 184 norovirus outbreaks, compared to only 9% of 1750 outbreaks due to other pathogens. However, this varied by food type. No infected food handlers were found in outbreaks related to oysters, while they were found in 47% of outbreaks due to other types of foods (Lopman et al., 2003). In a series of foodborne norovirus outbreaks in the U.S. reported from 2001 through 2008, with information on contributing factors, a food handler with direct contact with RTE foods was identified in 82% of outbreaks, and was reported to be the source of the outbreak in 53% (Hall, Eisenbart et al., 2012). Among those outbreaks for which the likely point of contamination was known, 97% of those due to leafy greens, fruits and nuts were judged to have been contaminated during final preparation or service, while 3% were due to contamination earlier during production and processing, and 100% of the shellfish-related outbreaks were due to contamination before final preparation (Figure 5- Food commodity and point of contamination implicated in reported norovirus outbreak involving simple foods, United States, 2001-2008 (Adapted from Hall, Eisenbart et al., 2012)).

Other studies suggest that the role of the ill food handler at point of preparation or service may be overestimated. In Europe, foodborne norovirus was evaluated by genotyping strains from feces, foods and oysters, which suggested that 25% of the outbreaks attributed to food handlers

were more likely due to contamination occurring earlier in the food chain (Verhoef et al., 2010). Norovirus genotyping may help exclude local food preparers as a source for foodborne illness if the outbreak strain in one location genetically matches others traced to the same food in distant locations.

Data on the prevalence of norovirus in foods are limited and difficult to interpret due to a lack of standardized methods and of a means to determine virus infectivity. Contamination in foods may be non-homogenous and at low concentrations (D'Souza et al., 2006). In the U.S. validated methods are currently available only for shellfish (Woods and Burkhardt, 2011), although investigational methods have been developed for produce and some other foods (Sair et al., 2002; Stals et al., 2011; Baert et al., 2008). ISO (International Organization for Standardization) Certified methods are now available although they have largely not been validated in the U.S (ISO, 2013a; ISO, 2013b). Standardized and validated methods for norovirus detection in produce are needed to accurately monitor the frequency of norovirus contamination during preharvest, harvest, and postharvest phases to direct safer handling practices (Mattison et al., 2010). Improved detection methods would enhance understanding of environmental persistence and the efficacy of disinfectants (see Chapter 3).

A market survey of live U.S. oysters found evidence of norovirus in 3.9% of samples using real-time RT-PCR (DePaola et al., 2010; Woods and Burkhardt, 2010). A recent two-year systematic study of oysters in the UK using a similar method found that 76% (643/844 samples) of oysters from 39 harvest sites were positive for norovirus genogroups I and II; all sites yielded at least one positive result (Lowther et al., 2012). One recent survey of packaged pre-washed leafy greens in Canada detected norovirus contamination by sequence conformation in 6% of retail samples (Mattison et al., 2010). In a recent survey of European produce, norovirus was detected using real-time RT-PCR in 28.2% of 867 samples of leafy greens, 33.3% of 180 samples of fresh fruits, and 50% of 57 samples of other produce (e.g., tomatoes and cucumbers) (Baert et al., 2011). Of these RT-PCR positive samples, only 7% were confirmed by sequencing, a result that illustrates the difficulty of interpreting RT-PCR findings in food.

These limited data suggest contamination may occur before the final preparation and service step. However, such possible contamination needs to be correlated with human illness data before concluding that much of sporadic illness is due to foods contaminated earlier in food production. (Hedberg, 2010).

The value of subtype-based surveillance

Use of standardized molecular genotyping methods and surveillance databases for comparison of outbreak strains hold great promise for improving our understanding of norovirus epidemiology. Systems such as CaliciNet in the United States and the global NoroNet surveillance system have demonstrated the potential utility of such systems in identifying emergent viral strains, confirming links between clusters of cases, and describing strain-specific patterns of transmission (Vega, Barclay et al., 2011; Verhoef et al., 2009; Kroneman et al., 2008). Currently, outbreaks of norovirus infection are almost always detected because a number of people become ill at once in one group. In the future, subtyping of sporadic cases may help identify more dispersed outbreaks when such surveillance is able to link individual cases in the

absence of a recognized outbreak. In addition, genotyping specimens from outbreaks can link together outbreaks that may have a common source. Currently such outbreaks are recognized if they are due to shellfish from one harvest site. In the future, with better genotype-based surveillance, other foods causing multi-site groups of outbreaks are likely to be recognized.

In the US, the association of a novel strain of GII.12 norovirus with several foodborne outbreaks illustrates how norovirus can be disseminated through a widely distributed food (Vega and Vinjé 2011). In Europe, genotyping helped find unsuspected connections between apparently separate outbreaks. For example, in 2005, investigation of a series of outbreaks in Denmark showed that they were all associated with the consumption of frozen raspberries imported from Poland; in 2006, a similar series of outbreaks in Sweden was linked to importation of frozen raspberries from China; and in 2009, outbreaks in Finland were linked to shipments of frozen raspberries from Poland (Falkenhorst et al., 2005; Hjertqvist et al., 2006; Maunula et al., 2009). Subtyping strains from these outbreaks suggested links among the outbreaks and supported the epidemiological link between illnesses and raspberries. Retrospective genotyping of outbreaks across Europe linked simultaneous outbreaks in different locations, few of which had previously been connected, and suggested that a common contamination source existed early in the food chain in 7% of outbreaks (Verhoef et al., 2011).

The European experience shows how systematic reporting and genotyping of norovirus outbreaks across a continent helps identify novel or rare genotypes linked to chains of transmission and contamination events far upstream from the final kitchen. Application of subtyping to characterize outbreak strains of norovirus may identify links between related outbreaks that were not previously suspected, indicating that the implicated food was contaminated before it was finally prepared in several separate kitchens. If greater use of molecular subtyping identifies more multi-site foodborne outbreaks, the impression that contamination almost always occurs in the kitchen might change.

Knowledge Gaps and Recommendations

<u>Improving estimates of public health burden and the fraction attributable to foods</u>

Although an increasing number of clinical and hospital laboratories offer testing for norovirus, laboratory diagnosis in the clinical setting is not routine and is largely restricted to research settings and outbreak investigations. Reliable population-based surveillance awaits rapid, accurate and inexpensive tools for clinical diagnosis.

- Develop and validate rapid, accurate and inexpensive tools for clinical diagnosis to conduct reliable population-based surveillance.

Current estimates of the norovirus disease burden in the US extrapolate from studies in other developed countries and illness surveys that are eight years old (Scallan et al., 2011) or from testing of limited collections of stools submitted for bacterial diagnostics (Hall, Rosenthal et al., 2011). Up-to-date estimates of the amount of AGE and prospective active surveillance in selected populations are needed to update the estimate of the etiological fraction for norovirus in the US.

- Conduct a new illness survey and prospective cohort studies to improve estimates of the burden of sporadic disease due to norovirus.

Because the frequency of norovirus disease fluctuates seasonally and from year to year, as new strains emerge, surveillance needs to be subtype-based (Zheng et al., 2010; Yen et al., 2011; Lopman et al., 2011; Hall, Lopman et al., 2013; Vega et al., 2014). Such data could help assess the effectiveness of specific interventions, such as food safety improvements or future vaccines, as well as the impact of emergent strains.

- Conduct sustained active surveillance in sentinel sites for sporadic cases, including subtyping to improve the accuracy and precision of these estimates, and to track trends in individual genotypes.

General estimates of the proportion of norovirus cases that are from food depends almost entirely on analysis of a series of reported outbreaks, and outbreak reporting depends greatly on the capacity of public health surveillance to diagnose and investigate all norovirus outbreaks, whether foodborne or not. This capacity has increased in the last decade, and the distribution of genotypes has changed, so that some caution is needed when combining the results of different series from different time periods (Widdowson et al., 2005; Vega et al., 2011).

- Identify comparable series of norovirus outbreaks from both foodborne and person-to-person transmission detected through robust public health surveillance in several nations to more accurately generalize the estimates.

Application of genotyping to outbreak surveillance is revealing great and growing diversity in type, with rapid emergence of new types and shifts in type–specific prevalence. Routine monitoring of these changes in the United States and in other countries using standard typing and nomenclature is needed to better understand these changes.

- Continue and expand genotype-based surveillance of outbreaks in CaliciNet, link those data to NORS, and compare genotypes of strains from outbreaks with those from sporadic cases.

Most of what we know about the epidemiology of norovirus infections comes from outbreak investigations. The quality of these investigations affects the robustness of inferences made from them. Many investigations and reports are incomplete. Limited public health resources and competing priorities curtail detailed investigations at the state and local levels, particularly of suspected person-to-person norovirus outbreaks that may not be investigated in depth by some public health agencies because prevention or control measures are routine.

- Expand the sentinel outbreak surveillance program NoroSTAT, so that representative data on outbreaks of all transmission modes are collected rapidly and systematically, and combined with genotyping data.

General outbreak control guidelines and specific recommendations for healthcare, food service, and cruise ship settings are based on the best available evidence but are limited by the lack of empirical data for many specific interventions (Hall, Vinjé et al., 2011; MacCannell et al., 2011; US FDA 2012 Food Code, CDC 2011 VSP, Harris et al., 2010, CIFOR 2013, CDC 2014 Norovirus working with food). More research is needed to determine the impact, feasibility, and cost-effectiveness of specific interventions.

- Evaluate the effectiveness of routine control recommendations by observing which control measures are implemented and comparing the subsequent course of the outbreaks.
- Evaluate the impact of susceptibility and infectivity risk factors for secondary household transmission following point source outbreaks to develop or improve prevention strategies.

Improving understanding of the foods that transmit norovirus and sites of contamination

Foodborne outbreaks provide critical data with which to understand the spectrum of foods involved. However, when a food is implicated, it is often difficult to determine whether the food was contaminated earlier in the food chain, or became contaminated after reaching the kitchen, because of an infected food handler, or cross contamination from other foods or food contact surfaces. Reliable information about sites of food contamination is rare. Outbreak investigations often do not indicate how or where an implicated food became contaminated.

- Collect more consistent and detailed environmental assessments through the new assessment tool, the National Voluntary Environmental Assessment Information System (NVEAIS), developed by CDC, and ensure these are linked to NORS and CaliciNet reports.
- Conduct traceback and root cause analyses during selected foodborne outbreak investigations to help clarify the role and mechanisms of upstream contamination in causing norovirus outbreaks.

Few food surveys exist demonstrating the frequency of contamination, and methods for detecting and subtyping norovirus in many foods remain to be developed and standardized. Such methods, particularly for fresh produce items, could assist in evaluating the frequency of contamination and in implicating a food vehicle.

- Develop standardized and sensitive methods to reliably detect norovirus in a variety of food matrices suitable for systematic surveys.

Improving understanding of microbiological characteristics and strain variation

Norovirus poses basic virological challenges. The inability to grow human noroviruses in cell culture (see Chapter 4) is a large obstacle, necessitating reliance on molecular methods to detect norovirus RNA. Without reliable means of defining infectivity outside of human-feeding studies, the detection of norovirus nucleic acid by PCR is difficult to interpret, as the viral sequence detected may not be part of an infectious particle.

- Develop a robust infectivity assay useable in surveys of foods and environment.

Genotypes vary in basic epidemiological characteristics, like seasonality and route of transmission, although there is some overlap. It is not known whether there is strain-to-strain variability in the ability of norovirus to persist in the environment or on foods, or to resist disinfectants.

- Develop strategies to make genotypic-specific comparisons of susceptibility to disinfectants, and persistence
- Consider genotype-specific assessments of risk and control.

Genotyping has advanced rapidly in the last decade, and has now been standardized both nationally (Vega et al., 2011) as well as internationally. Further uptake and use of these systems

will help identify links in real time between outbreaks and common sources, such as distributed food products.

- Integration of CaliciNet with NORS and more timely uploading of data into these systems are the next steps necessary towards improved national outbreak surveillance.
- Increase the collaboration with NoroNet laboratories by harmonizing methods to build a global norovirus surveillance network that will help identify new genotypes and new emerging (GII.4) viruses when and where they arise.

[Four norovirus contamination-point case studies to be included in separate boxes.]

1) Colorado River rafters travel downstream with packaged deli meats (Malek et al., 2009). In 2005, 57 rafters on 13 trips down river experienced diarrhea and vomiting that began ≤72 hours after launch, 96% of whom ate prepackaged deli meat (sliced, vacuum-packed and frozen) from one supplier produced on one day. Identical norovirus sequences were identified in stool specimens from ill rafters on five different trips. Norovirus sequence was found in unopened deli meat by PCR. It was concluded that the ready-to-eat meat was contaminated during slicing by a worker with diarrhea and vomiting the day before he handled it.

2) Norovirus goes airborne (Repp and Keene, 2012).
A reusable grocery bag stored in a hotel-room bathroom was the means by which nine players of a traveling soccer team contracted norovirus. A player became ill, vomited into the toilet in the hotel bathroom, and then went home without seeing other teammates. In the bag, stored in that bathroom were team snacks comprised of chips, grapes and cookies, which were eaten by team members the next day. The virus presumably was aerosolized in the bathroom and contaminated the bag and its contents.

3) Misadventures in oyster-harvesting (Kohn et al., 1995).
Discharged stools or vomit from ill oyster harvesters fouled a Louisiana oyster bed. A multistate outbreak of 70 norovirus infections was linked to oysters harvested from one harvest site on one day. Crews from 22 oyster boats in the area reported routine overboard disposal of sewage. One oyster harvester who reported diarrhea and vomiting shortly before the implicated harvest had serologic evidence of recent infection. Infected feces or vomit going overboard can contaminate large volumes of shallow water, and oysters can bioaccumulate nearly 100-fold greater concentrations of virus in their tissues than virus levels in the surrounding waters.

4) Loaded imported raspberries (Falkenhorst et al., 2005).
A cold dish prepared from frozen raspberries caused six European norovirus outbreaks in 2005. All told, more than 1,000 people became ill with several different norovirus strains found in raspberries originally grown in 2004 on several different small farms in Poland, then purchased, frozen and packaged before export. Fecal-contaminated irrigation water, infected farm workers and unsanitary processing at the plant were suggested as contamination possibilities.

Figures: (See separate Document of figures)

Figure 1): Estimates of the annual burden and individual lifetime risks for norovirus disease in the United States, 1997-2009 (Adapted from Fig 3, Hall, Lopman et al., 2013).

Figure 2): Number of reported outbreaks of norovirus infection by month and mode of transmission, 2009-2012, United States (Hall, Wikswo et al., 2014).

Figure 3): Incidence of outpatient visits, emergency department visits and hospitalizations due to norovirus infection by age, United States, 1996-2009 (Adapted from Hall, Lopman et al., 2013).

Figure 4): Attribution of norovirus illnesses to specific food commodities based on outbreaks reported 1998-2008, in which simple and complex foods were implicated (Adapted from Painter et al., 2013 - Online Table 4).

Figure 5): Food commodity and point of contamination implicated in reported norovirus outbreak involving simple foods, United States, 2001-2008 (Adapted from Hall, Eisenbart et al., 2012).

Table 1. Estimates of the general population incidence of norovirus disease in the United States and other countries (adapted from Hall, Lopman et al., 2013).

Country	Data period	Method	Incidence per 100	Reference
United States	1997-99	Etiologic %	8.6	Mead et al., 1999
United States	2000-06	Etiologic %	7.0	Scallan et al., 2011
United States	2004-05	Prospective cohort	6.5	Hall, Rosenthal et al., 2011
England and Wales	1993-96	Prospective cohort	4.5	Wheeler et al.,1999 Phillips et al., 2010
England and Wales	2007-09	Prospective cohort	4.7	Tam et al., 2012
The Netherlands	1999	Prospective cohort	3.1	DeWit et al., 2001
The Netherlands	2009	Etiologic %	3.8	Verhoef et al., 2013
Canada	2006	Etiologic %	10.4	Thomas et al., 2013

Table 2. Epidemiological characteristics of outbreaks of norovirus infection caused by genotype GII.4 strains and nonGII.4 strains (from Desai et al., 2012; Leshem, Wikswo et al., 2013; Leshem, Barclay et al., 2013; Matthews et al., 2012; Vega et al., 2014).

	GII.4	**Non – GII.4**
Seasonality	Winter	Spring –summer or non-seasonal
Setting	Health care facilities	Restaurants, schools, other non-health care facilities
Most likely transmission	Direct person-to-person	Foodborne
Ages affected	> 65 years	< 65 years
Severity	Higher rates of death	Lower rates of severe outcomes and hospitalization

Chapter 3: NOROVIRUS AND CONTROLS (QUESTIONS 3, 4, 6, AND 7)

Retail and foodservice employees represent the largest workforce in the food industry. This Chapter has a primary focus on the retail and foodservice industries but, where relevant, food production and processing are considered. Foods are commonly handled by human hands and then are consumed or sold soon after preparation. Norovirus presents challenges for the retail and foodservice industry due to several factors: it is highly contagious; has a low infectious dose; has multiple modes of transmission; is stable in the environment; is more resistant to commonly used cleaning and sanitation methods than most other pathogens (Kingsley, Vincent et al., 2014); and food workers can have asymptomatic infections. Foods most often associated with outbreaks at foodservice and retail food establishments include raw shellfish (i.e., oysters, clams), and any ready-to-eat foods that require extensive handling, such as salads, peeled fruits, deli-sandwiches, finger foods, hors d'oeuvres, and dips. The behavior of food handlers in retail and foodservice establishments thus represents a significant risk for the spread and transmission of norovirus.

As noted above, the great majority of reported foodborne outbreaks of norovirus infection occur in commercial food preparation settings. In a quantitative mathematical exposure model estimating the transmission of norovirus in retail food preparation, the modelers noted that hand-washing compliance and gloving would be the most effective factors in controlling contamination of food products (Mokhtari and Jaykus, 2009). Additionally, the restroom environment (used by employees and patrons alike) was judged to be the primary focus for norovirus transmission even if no employees harbored the virus. All of these factors contribute to an elevated risk for norovirus contamination of foods in retail and foodservice establishments. These factors should be considered for the pre-harvest and food processing industries as well.

Charge 3: Are there conditions or food matrices for which the current minimum cooking temperatures in Part 3-4 of the FDA 2009 Food Code might be inadequate for destruction or inactivation of HuNoVs?

Part 3-4 of the FDA Food Code ("Food Code") addresses destruction of organisms of public health concern. Subparts 3-401 through 3-404 describe parameters for the destruction of organisms of public health concern by cooking, freezing, reheating and other methods. When adopted, the Food Code establishes requirements for the cooking of raw animal and plant foods, and for the reheating and hot holding of foods that require temperature control for safety. Minimum cooking temperatures range from 54°C (129.2°F) to 74°C (165.2°F) depending on the type of raw animal food and, in some cases, method of preparation.

These minimum cooking times and temperatures in the Food Code are largely based on the adequate destruction of bacterial pathogens and not viruses. Therefore, there are still many unknowns relative to the cooking temperatures and times that would be required for different foods prepared at retail food establishments and restaurants to destroy HuNoV. Thus some of the Food Code cooking parameters (US FDA, 2013 Food Code) may be insufficient for norovirus inactivation because, under laboratory conditions, norovirus seems to exhibit heat resistance that exceeds the cooking temperature recommendations.

Published information discussed below was found related to heat inactivation of viruses for temperatures higher than those recommended in the Food Code or for foods that would not be required to be cooked at retail. The available research also did not always record the internal temperatures of food. Although some data exist on thermal inactivation of surrogates, it is unknown how these data translate to HuNoV.

While several conditions listed in the Food Code could be inadequate for complete destruction/inactivation of HuNoVs, there are virtually no epidemiological data linking properly cooked foods, which have not been handled after processing, to norovirus illness. Published results from research vary based on the type of food, the surrogate, the method, and the strain type used. Mormann et al. (2010) used HuNoV genotype II for heat inactivation studies and determined log inactivation based on PCR reduction. They found significant reductions in virus titers in high heat processes conditions typically used in baking, cooking and roasting. However more moderate heat treatments (e.g., pasteurization) were less effective at inactivating HuNoVs in food matrices or on food surfaces. Dancho et al. (2012) showed no inactivation of HuNoVs below 60°C (140°F). Bozkurt et al. (2013) compared heat inactivation of FCV and MNV-1. MNV-1 was more sensitive than FCV-F9 up to 65°C (149°F). At 72°C, FCV-F9 was slightly more susceptible to heat inactivation. Topping et al. (2009) found that NoV GII.4 was more resistant than FCV and that it may be resistant to "typical" pasteurization temperatures 71.6°C/160.9°F 15s for milk or 70°C/158°F for 2 min cook/chill food processing). Seo et al. (2012) studied virus inactivation and reported that MNV was heat sensitive because it was rapidly inactivated at temperatures above 60°C (140°F). Conversely, it should be noted that several of these studies measured HuNoV inactivation using PCR reduction that did not measure virus particle infectivity. As such, some of these studies may have underestimated the reductions in infectious HuNoV.

What temperature must molluscan shellfish be heated to in order to inactivate HuNoVs and is this temperature reached when the product is prepared in traditional ways such as steaming for culinary (but not food safety) purposes?

The thermal sensitivity of HuNoV in molluscan shellfish has not been determined. The United Kingdom set standards for commercial shellfish temperatures based on research demonstrating a 4-log reduction of hepatitis A (HAV) in molluscan shellfish after holding at an internal temperature of 90°C (194°F) for 1.5 minutes (Lees, 2000). As noted by NACMCF in "Response to Questions Posed by the Food and Drug Administration and the National Marine Fisheries Service Regarding Determination of Cooking Parameters for Safe Seafood for Consumers", "this temperature and time combination likely will achieve an equivalent or greater level of reduction for HuNoV, although this assumption has not been confirmed." Moreover, compliance with this temperature and time combination may not be optimal for consumer acceptance because it is likely that the product will be overcooked from an organoleptic perspective (NACMCF, 2008).

The consumer practice of steaming mollusks just long enough to open the shells may cause some reduction but may not completely eliminate the virus. Holding mollusks under steam for longer periods of time is expected to have a more lethal effect on HuNoVs (NACMCF, 2008).

Is foodborne transmission of HuNoVs affected by the holding temperature of food?

The FDA Food Code requires that foods requiring temperature control for safety be maintained at 5°C (41°F) or less, or at 57°C (135°F) or above during storage and display in a food establishment. While surrogate data imply transmission may be affected by hot holding, this has yet to be determined for HuNoV. It is likely that HuNoV can remain infectious indefinitely at cold temperatures (Bertrand et al., 2012). The cold and hot holding temperatures in the Food Code are primarily established to minimize or prevent bacterial growth and therefore may not be applicable to norovirus since it does not grow in food, as it requires a human host to multiply.

Does recontamination of cooked food present increased risk for transmission?

Post-lethality contamination of ready-to-eat foods, interpreted as recontamination, increases the risk from numerous pathogens, including HuNoVs. The risk is likely proportional to the number of infectious units present (Malek et al., 2009). RTE food items that are likely to be handled by human hands are at risk for contamination by norovirus. Transmission of HuNoVs to food, in retail and foodservice settings, may occur either through ill food workers, cross-contamination of infected food and food contact surfaces, or through indirect contamination with vomitus from customers (Mathijs et al., 2012).

Considering only preparation and point of service, how likely is food to be contaminated at this phase?

Among foodborne norovirus outbreaks, for which the likely point of contamination is known, 85% involve contamination during preparation or service (Hall, Eisenbart et al., 2012). Since humans are the primary carrier of norovirus, food handlers in restaurants and retail settings should be considered as one of the primary sources of food contamination during preparation at the point of service. This suggests that effective preventive controls targeted to retail establishments and foodservice could have a large impact on minimizing illnesses from HuNoV. As an example, the development of effective education and training programs for food handlers in retail and foodservice industries should be a priority.

To illustrate this point, according to the National Restaurant Association (NRA), there are 13.5 million restaurant employees employed in the U.S. (NRA, 2014). The estimated diarrheal illness rate is 0.6 illnesses per person per year which equates to 8.1 million total illnesses per year for these employees (Jones et al., 2007). Norovirus accounts for about 16% of AGE illnesses in the U.S. (Hall, Lopman et al., 2013); therefore NACMCF estimates 1.3 million cases of norovirus infection occur among restaurant employees each year. In a 2005 survey, 5% of food workers self-reported working while ill with vomiting or diarrhea (Green et al., 2005). Using the estimate of 1.3 million above, this would result in approximately 65,000 norovirus-infected restaurant employees that continue to work while ill each year.

The Food Marketing Institute (FMI) estimates that there are 3.4 million grocery store employees that work in over 37,000 supermarkets (FMI, 2012), using extrapolated data and the same assumptions as above, NACMCF estimates 325,000 cases of norovirus infection among grocery employees each year which could result in more than 16,000 norovirus-infected grocery employees that continue to work while ill each year. Deli operations and the bakery are the most likely areas for food worker hand contact with foods that would contribute to the greatest risk of norovirus transmission.

Exclusion and restriction of ill food handlers as identified in the Food Code is envisioned as one of the most important mitigation strategies for controlling the transmission of HuNoV (Hall, Vinjé et al., 2011). Not all infected food workers are symptomatic and many HuNoV foodborne outbreaks are initiated by asymptomatic or post-symptomatic food workers transmitting the virus during food handling procedures. Therefore, exclusion and restriction of ill food handlers, while an important mitigation strategy, is not always effective unless additional barriers to bare hand contact and transmission are in place. Further awareness, education and training for managers in charge and for food handlers are needed to realize the optimal benefit of this mitigation strategy.

Recommendations:

- Conduct research on how HuNoV behaves in typical food service cooking protocols such as those referenced in the Food Code.
- Reinforce preexisting NACMCF recommendations to avoid bare hand contact with RTE foods. (NACMCF, 1999)
- Conduct a risk assessment to determine the cost benefit analysis of excluding ill food workers.

Charge 7: Discuss the possible impact and burden of HuNoV illness if the preventive controls such as those listed in the Parts 2-2, 2-3, and 3-3 of the 2009 FDA Food Code for retail operations were also required of producers of RTE foods.

Parts 2-2, 2-3 and 3-3 of the Food Code address employee health, personal cleanliness and protection from contamination after receiving, respectively. The term “producer” is defined as any segment of the food industry that handles, prepares, manufacturers, and/ or packs ready-to-eat foods other than retail or foodservice operations. The RTE food industry is quite broad and could include complex prepared foods (such as sandwiches and deli salads) as well as foods that

may be handled and packed during harvest as ready-to-eat foods (such as berries and salad ingredients).

There are regulations and recommendations for the handling of ready-to-eat foods that other segments of the food industry use. Food manufacturers are required to comply with the current Good Manufacturing Practices (cGMPs) in 21 CFR 110 (US FDA, 2013 - 21CFR 110). Good Agricultural Practices (GAPs) (US FDA, 2008) provide safe food handling recommendations for on-farm growing and harvesting operations, and these guidelines are used primarily for fruit and vegetable operations. Compared to the cGMPs, and GAPs, the Food Code provides a more complete source of recommendations for handling ready-to-eat foods, especially related to exclusion and restriction of ill food employees, and disease control. Food production (farms) and food manufacturing industries may benefit by some of the provisions provided in the Food Code, and they may want to consider recommendations that are provided in these sections of the Food Code to develop guidance for their industries. More research and information specific to the control and prevention of viral foodborne pathogens including norovirus are needed to substantiate the effectiveness of prevention and controls provided in the Food Code.

The retail and foodservice industry has adopted a number of key strategies for the overall control of norovirus (FMI, 2010; NRA, 2007). The Committee believes that these same strategies would be helpful for food production and food manufacturing operations. Overall strategies used in prevention and control of norovirus at retail and foodservice include:

- Handwashing and good personal hygiene
- Prohibiting bare hand contact with RTE food items
- Removing/Excluding food workers that are infective
- Applying effective sanitation and disinfection, particularly in food preparation areas and in bathrooms
- Applying proper cooking parameters (e.g. time and temperature)
- Purchasing food from an approved source

In cases where there is a vomiting and/or diarrheal event, the retail and foodservice industry uses additional strategies for control of norovirus that include:

- Reducing airborne transmission, and treat vomitus and feces as infectious material
- Cleaning staff should use barriers, such as face masks, gloves, and aprons
- Disposing of materials used to clean-up vomiting incident, and thoroughly disinfect the area

Recommendations for HuNoV control in the FDA Food Code that may impact producers of ready-to-eat foods

Part 2-2 of the 2013 FDA Food Code addresses symptoms and exclusions for food workers. It is intended to identify when a food employee should be excluded from working in a retail or foodservice establishment. Subparagraph 2-201.11 (A) (2) “Reportable Diagnosis” requires food employees to report illness diagnosed by a health practitioner due to norovirus. Subparagraph 2-201.11 (A) (4) “Reportable History” requires food workers to report if they have been exposed to norovirus within the past 48 hours. Subparagraph 2-201.12 (A) (2) “Exclusions and Restrictions” excludes food employees from working if they are symptomatic with vomiting or

diarrhea or diagnosed with an infection from norovirus. Paragraph 2-201.12 (D) "Exclusions and Restrictions" restricts a food employee from working if diagnosed with an infection from norovirus and is asymptomatic. Paragraph 2-201.13 (D) "Removal, Adjustment, or Retention of Exclusions and Restrictions" outlines conditions under which a previously excluded food employee may be reinstated following a norovirus diagnosis including obtaining written medical documentation from a health practitioner stating that the food employee is free of a norovirus infection, or the absence of symptoms (vomiting and diarrhea) and more than 48 hours have passed since the employee became asymptomatic, or the employee did not develop symptoms and more than 48 hours have passed since diagnosis. This section of the Food Code is very prescriptive for the identification of ill food workers and workers that are infectious (or carriers) of norovirus and other identified foodborne pathogens. While implementation may be difficult if food workers do not comply or food managers cannot identify symptoms of the disease, if followed correctly these provisions should result in significant reduction in norovirus transmission in retail food stores and restaurants; however, it should be recognized that shedding probably continues for about 2 weeks after symptoms resolve.

Section 2-301.14 of the Food Code provides guidance for "When to Wash". Food employees shall clean their hands:

- After touching bare human body parts other than clean hands and clean, exposed portions of arms;
- After using the toilet room;
- After caring for or handling service animals or aquatic animals;
- After coughing, sneezing, using a handkerchief or disposable tissue, using tobacco, eating, or drinking;
- After handling soiled equipment or utensils;
- During food preparation, as often as necessary to remove soil and contamination and to prevent cross contamination when changing tasks;
- When switching between working with raw food and working with ready-to-eat food;
- Before donning gloves for working with food; and
- After engaging in other activities that contaminate the hands.

The Food Code also recommends that "food employees clean their hands and exposed portions of their arms, including surrogate prosthetic devices for hands or arms for at least 15-20 seconds, using a cleaning compound in a handwashing sink." CDC suggests that handwashing is the single most important procedure for preventing the spread of infection (CDC, 2013). For norovirus this is especially true since the virus can be spread by contaminated hands of food workers to foods. Identification of when to wash hands and for how long certainly will have some minimizing effect of norovirus transmission from food workers in retail establishments and restaurants; however, the true level of impact and effect is unknown. Data used for making recommendations for when to wash and for how long to wash were obtained from studies using bacterial pathogens. Most of the related studies that would be more specific to norovirus control have been done using surrogate organisms, so the true impact of handwashing for norovirus control is unknown.

Part 3-3 of the 2013 Food Code deals with ”Protection from Contamination after Receiving” The most relevant section of the code for the control of norovirus is Section 3-301.11 – “Preventing Contamination from Hands.” When an infected food worker is shedding the virus at high levels, handwashing may not be completely effective in removing the virus contamination from their hands. The provision in the Food Code under 3-301.11 provides an additional barrier for the transmission of noroviruses from contaminated food workers hands, by requiring the use of utensils, deli paper, or gloves in handling ready-to-eat foods. In a quantitative microbial risk assessment on the transmission of norovirus in the retail environment, Mokhtari and Jaykus (2009) found that gloving compliance, how often gloves are and should be changed, was one of the most effective mitigation strategies in controlling contamination of food products when practiced simultaneously with handwashing.

As a part of Section 3-302.11, (“Packaged and Unpackaged Food - Separation, Packaging, and Segregation”), recommendations are provided for the separation of raw animal food, which includes fish for sushi or molluscan shellfish, and ready-to-eat food. Since norovirus can be found in raw seafood and can be transmitted to other foods, separation of raw and ready-to-eat foods could certainly be an important strategy for minimizing contamination. Section 3-302.15 of the Food Code addresses “Washing Fruits and Vegetables” and recommends, with exceptions, that whole, “raw fruits and vegetables shall be thoroughly washed in water to remove soil and other contaminants before being cut, combined with other ingredients, cooked, served, or offered for human consumption in ready-to-eat form.” While the Food Code does not specifically recommend washing raw fruits and vegetables as a control strategy for microbial reduction, many studies have been published which demonstrate a potential reduction in levels of foodborne pathogens, such as *E. coli*, *Salmonella* and *Listeria*, however not enough science has been developed to show the impact of produce washing on the removal and control of norovirus.

Recommendations for HuNoV control from the retail and foodservice industry that may impact producers of ready-to-eat foods

The retail and foodservice industries have worked to reinforce and perhaps exceed the recommendations in the Food Code, for focused control of HuNoV from the food handler. As an example, The National Restaurant Association (NRA, 2007) has identified and developed training materials that focus on three core preventive measures that need to be in place for control of norovirus in restaurants that include:

- Reinforcing proper handwashing as a key to preventing the spread of norovirus.
- Developing an employee illness policy to help prevent (exclude) infected food handlers from contaminating food.
- Having a certified kitchen manager on every shift to reinforce food safety and to encourage compliance with exclusion/restriction procedures.

The Food Marketing Institute (FMI) assembled a strong science-based team to establish specific recommendations for control of norovirus in retail food establishments. Some of the key items and action for specific control of norovirus are shown in the table below (FMI, 2010). Many of these action steps are more detailed and more prescriptive compared to recommendations found in the Food Code, and should be considered for other segments of the food industry that handles, prepares, manufacturers, and/ or packs ready-to-eat foods.

Item	Action
Possible norovirus vomitus or fecal incident within food preparation department.	Immediately stop all food preparation and service, secure area and implement clean-up. and disinfection procedures
Possible norovirus vomitus or fecal incident within public, dining, or non-food preparation areas.	Secure area, immediately stop all food preparation and service.
Any 'open or in-use' food, ice or single service items within defined area directly contaminated	Discard
Any 'open or in-use' food, ice or single service items within defined area that could have been contaminated by airborne (aerosolized) droplets.	Secure area, implement clean-up and disinfection procedures.
Exposed food preparation equipment and utensils within defined area.	Assess, clean and disinfect as needed.
Non-food contact surfaces within defined area.	Assess, clean and disinfect as needed.
Linens (including clothes, aprons, wiping cloths, napkins, table cloths, etc.) within defined area of incident.	Thoroughly wash using 'hot' water cycle – at temperature >160°F (71°C), >25 minutes.
Carpeted areas within defined area of incident.	Steam clean at a minimum of 140°F (60°C).
Air ventilation systems within adjacent areas (i.e., exhaust hoods, vents in restrooms).	Implement clean-up and disinfection procedures for exterior of ventilation systems. Assess if additional disinfection for interior of system would be necessary.

Recommendations:

The level of impact and burden of adapting more specific HuNoV control measures from the FDA Food Code and/or from industry guidance is not possible to quantify due to the limitations in available science. Clearly, more studies need to be performed which can better measure impact and burden of control measures against HuNoV. However, it is clear that there are more extensive control measures that have been developed for retail and foodservice establishments from the FDA Food Code, and from industry associations such as the NRA and FMI. These control measures are far more specific to control HuNoV as compared to provisions contained in GAPs and GMPs. Producers of ready-to-eat foods should consider information provided in the FDA Food Code and from the NRA and FMI when establishing more specific control measures for HuNoV.

Charge 6: What interventions are available, or might be available in the near future, to reduce or eliminate the likelihood of HuNoV contamination for at-risk foods? Please consider interventions based on:

- ***Separating ill or infectious persons (including asymptomatic) from food/time interval for when ill food workers can return to work;***

- ***Reducing contact with contaminated hands;***
- ***Cleaning of hands and cleaning and sanitizing food contact surfaces;***
- ***Using appropriate procedures in response to incidents of vomiting or diarrhea that occur at food establishments;***
- ***Focusing on interventions upstream from final preparation and final service.***

Various prevention strategies have to occur at different stages for different food products: at pre-harvest for mollusks and fresh produce destined for raw consumption; at harvest, packing, and storage for fresh fruits and vegetables; and at post-harvest for prepared ready-to-eat foods (FAO/WHO, 2012). The Committee recommends that guidance from Good Agricultural Practices (US FDA, 1998; EFSA, 2014) and the National Shellfish Sanitation Program (US FDA, 2011 NSSP) could play a significant role in potentially reducing the transmission of HuNoV. The 1998 FDA *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* provides guidance on safe handling practices for pre- and post-harvest handling of fresh produce. While not specifically targeted to viral hazards, Norwalk and Hepatitis were considered, and many of the recommendations are expected to reduce risk of HuNoV transmission. For example:

- Water: "Review existing practices and conditions to identify potential sources of contamination….Agricultural water can become contaminated, directly or indirectly, by improperly managed human or animal waste. Human contamination may occur from improperly designed or malfunctioning septic systems and sewage treatment facility discharges such as combined sewer overflows and storm sewer overflows."
- Manure and municipal biosolids: "Growers using biosolids must first meet the requirements of [40 CFR part 503] and then comply with any additional state requirements" for treatment of manure and municipal biosolids.
- Worker health and hygiene: "Infected employees who work with fresh produce increase the risk of transmitting foodborne illnesses… Train all employees to follow good hygienic practices… The importance of handwashing… The importance of proper handwashing techniques…"
- Sanitary facilities: "Systems and practices should be in place to ensure safe management and disposal of waste from permanently installed or portable toilets to prevent drainage into the field… Poor management of human and other wastes in the field can significantly increase the risk of contaminating produce."

Additional industry and FDA fresh produce guidance documents for leafy greens, tomatoes and melons, among others, cite similar safe handling practices (United Fresh Produce Association guidelines 2005, 2006 & 2008).

<u>The National Shellfish Sanitation Program (NSSP) (US FDA, 2011)</u> provides guidance to culture and harvest of molluscan shellfish. This program classifies harvest areas based in part on fecal coliform levels in harvest waters as an assessment for fecal impacts on shellfish growing areas. This classification system does not directly measure HuNoV but does essentially provide a basic assessment as to the sanitary quality of harvest waters and mandates suitable harvest areas and prohibited areas. The NSSP makes specific recommendations for handling and holding of shellfish during and after harvest.

Other Recommendations (specifically for Post-Harvest):

1. Water used during packing, storage, and distribution of any food material should be suitable for its intended use so that food safety is not compromised.
2. Cleaning, maintenance and personnel hygiene at such facilities should follow procedures outlined for food establishments, such as those outlined in the FDA Food Code (e.g., Chapters 2, 3, and 4), on proper sanitation procedures and proper personal hygiene at all times.
3. Food sources should be protected from any contamination, notably fecal and vomitus.
4. As stated previously, exclusion and/or separation of ill food handlers from direct food handling could serve as key intervention strategy in preventing spread of HuNoV.
5. Management of vomiting or feces incidents, and effective cleaning and disinfection are emphasized as interventions in avoiding wide spread dissemination of HuNoV.
6. Management of cleaning and disinfection of restroom facilities should be a focus in potentially reducing the spread of HuNoV.

What interventions might be available in the near future?

For some foods, high pressure processing is a promising technology to control norovirus while retaining taste, flavor texture and nutritional value (Kingsley, 2013). A recent volunteer study indicates that HPP-treated oysters are well accepted by oyster eaters when treated at pressures that are known to inactivate human norovirus (Kingsley, Kuhn et al., 2014).

Several studies have been done with irradiation and ultraviolet radiation for inactivation of viruses; results indicate that their utility is limited. (Praveen et al., 2013; Sanglay et. al., 2011; Feng et al., 2011). Although these interventions cannot completely eliminate the risk of norovirus, infection risks may be reduced.

There are a multitude of intervention technologies being considered for foodborne illness agent control; examples include plasma (charged particles /ionized gas/fourth state of matter), electrolyzed water, and chlorine dioxide. Efficacy of these technologies against norovirus should be considered with an eye towards the type of food, the location of virus in food and the effectiveness of technology against the virus.

Charge 4: What factors most significantly affect the efficacy of removal and/or inactivation of HuNoVs from surfaces and hands when using common cleaning and sanitizing practices?

Noroviruses are non-enveloped single-stranded RNA viruses that are difficult to inactivate. Non-enveloped viruses contain no lipids, making them generally resistant to organic solvents such as alcohols and chloroform. While soaps and detergents may be effective surfactants for facilitating removal of viruses, these non-enveloped viruses are largely resistant to inactivation by soap and detergents. Robust inactivation requires chemically reactive compounds (e.g., iodine, chlorine). They also tolerate low and high ionic strength environments such as fresh and salt water. HuNoV is resistant to temperatures as high as 60°C (140°F), can persist for long periods at temperatures below room temperature, and are resistant to extremes in pH.

Hand Hygiene

Proper handwashing is critical for preventing HuNoV transmission and infection (Hall, Vinjé et al., 2011). After illness, infected persons typically shed the virus for 20 to 40 days (Bok and Green, 2012). Hands can pick up infectious viruses from contaminated surfaces. Sattar (2004) therefore emphasized frequent handwashing as well as cleaning and disinfection of frequent hand contact surfaces.

Soap and water vs. hand sanitizers. For the purposes of this document, the Committee defines handwashing as the use of soap and water combined with mechanical action for at least 15-20 seconds (rubbing hands together to create friction) followed by hand drying (NRA, 1995; EFSA, 2011; Hall, Vinjé et al., 2011). A quantitative exposure model published for transmission of NoV in retail food preparation suggested compliance with handwashing is an effective way to control contamination of food products (Mokhtari and Jaykus, 2009). Hand sanitizers should not be considered an effective method of handwashing and they should not be considered as a substitute for soap and water washing because their ability to inactivate HuNoV has not been defined or determined (NRA, 1992; Sickbert-Bennett et al., 2005; Liu et al., 2012; Kampf et al., 2005; Macinga et al., 2008; Lages et al., 2008; Park et al., 2010; Hall, Vinjé et al., 2011). Extensive research has been published related to the ability of soaps, antibacterial soaps and hand sanitizers to remove and/or inactivate HuNoV surrogates (i.e. MNV and FCV). Hand care products cannot list claims against norovirus in the U.S. because viral claims are not currently recognized by the FDA (US FDA, 1994). In Canada and some European countries, norovirus hand antiseptics may be cleared based on norovirus surrogate testing data. Several studies have reported that a simple water rinse, or use of antibacterial hand soap, was more effective than alcohol-based sanitizers for reducing viruses on hands (Ansari et al., 1988; Lin et al., 2003: Mbithi et al., 1993; Liu et al., 2010). Although water washing, with or without soap, may remove organic matter and many viral particles, it cannot be relied on to completely eliminate a virus which many be shed in titers of up to 10^{11} genomic copies per gram of stool (Atmar et al., 2008). While numerous studies evaluating the utility of sanitizers against surrogates such as FCV and MNV have been performed, these have not been validated for HuNoV and there is no assurance that hand sanitizers will inactivate HuNoV and are best used as an adjunct with proper and effective hand washing.

Drying of hands. The use of cloth hand towels is not recommended as there is the potential for cross-contamination with retractable cloth dispensers. While air dryers are often considered a sanitary method for drying hands, they are often slow, potentially causing employees and patrons to wipe their hands on their clothes. Disposable towels are recommended for drying hands.

Recommendations:

- Effective hand washing is an important part of a norovirus control program.
- Hand sanitizers cannot be relied on to effectively inactivate norovirus.
- In line with other countries and other hand care products, FDA should consider a less rigorous or expedited process for validation of virucidal claims on hand care products.

Environmental Cleaning and Disinfection

Hard Surfaces

Hard surfaces contaminated with soils that contain norovirus, such as feces and vomit, or those that are in contact with contaminated hands, food, and water need to be thoroughly cleaned and disinfected. Special attention should be paid to surfaces that may be highly contaminated such as those in bathrooms. More people using a communal bathroom increases risk of norovirus transmission (Ho et al., 1989).

Use of chemical disinfectants is one of the key approaches to interrupt the spread of viruses from contaminated environmental hard surfaces; it takes a stronger chemical (i.e., a disinfectant) than a sanitizer for norovirus inactivation. Disinfectants with claims against norovirus are regulated by the EPA and per their requirements, need to demonstrate efficacy (EPA, 2006). Currently, feline calicivirus is the only norovirus surrogate accepted by the US EPA for disinfectant claims. Recent research on comparing four different cultivable surrogate caliciviruses in response to different inactivation and disinfections treatments concludes that multiple surrogate viruses, such as murine norovirus and Tulane virus, rather than FCV alone, should be used to assess the efficacy of disinfectants (Cromeans, 2014).

Based on these tests, disinfectants with such claims include recommended concentrations and exposure times that must be followed for effective inactivation. It is a violation of Federal law to use an EPA-regulated product in a manner inconsistent with its labeling for viral inactivation, but there are pros and cons associated with many products. Doultree at al. (1999) tested several different disinfectants against FCV. The results demonstrated that high concentrations (1000 ppm) of freshly reconstituted hypochlorite solution applied for a minimum of 10 minutes were required to inactivate FCV. This is consistent with findings reported by Weber et al., 2005; Johnston, et al., 2007; Cheng et al., 2006; Stevenson et al., 1994; and Park and Sobsey, 2011. However, the application of a bleach solution is not appropriate for all situations due to discoloration (bleaching) and pitting of surfaces. Doultree at al. (1999) reported that glutaraldehyde and iodine-based products were also effective at inactivating FCV; however due to concerns with toxicity for the former and staining of treated surfaces for the latter, there may be practical limitations. Products containing phenolic compounds (including triclosan and quaternary ammonium compounds) were less effective against non-enveloped viruses (i.e. HuNoV); however, there are newer EPA-registered phenolic and quaternary ammonium products with validated claims against norovirus (US EPA, 2009).

Cleaning prior to disinfection is critical because the presence of organic materials (e.g., stool or vomitus) can dramatically reduce chlorine disinfectants effectiveness (Barker at al., 2004). Based on surrogate experiments, manual dishwashing and low temperature ware-washing machines may not be effective for complete inactivation of HuNoV. Studies demonstrated reductions of up to 3 logs (Feliciano et al., 2012); risk assessments need to be done to gauge whether this is effective enough. However, there are currently no epidemiological data linking clean wares to norovirus illness.

<u>Soft Surfaces</u>

Disinfection of soft surfaces and fabrics is difficult to achieve through normal cleaning. Chadwick et al. (2000) recommended steam cleaning for carpets and soft furnishings providing they are heat tolerant; this should be done where chemical disinfection is not appropriate. There have been no studies on the effectiveness of laundry products or procedures against norovirus,

but there are no epidemiological data linking properly laundered linens to norovirus illness. It is expected that high temperature laundry procedures and those using bleach will reduce norovirus infectivity.

Other Considerations for Cleaning and Disinfection Programs

Comprehensive training should be provided to employees, including housekeeping and janitorial staff, and managers. Topics for training include proper and effective hand hygiene, the potential sources and routes of transmission of viruses, the incubation periods and duration of virus shedding even after recovery from clinical symptoms; the possibility of co-worker and family infectivity and asymptomatic shedding; the infectivity and potential for contamination of vomit and diarrhea; and cleaning and disinfection of contaminated surfaces (EFSA, 2012).

Proper procedures are only part of an effective sanitation program. Identifying and containing the area(s) of contamination presents a significant challenge to effective cleaning and decontamination during and after an outbreak, particularly one that involves diarrhea and/or vomitus. Results of investigations of outbreaks of viral gastroenteritis have implicated airborne transmission and aerosolized droplets of vomitus as a likely source of person-to-person transmission (Marks et al., 2000; Heun et al., 1987; Ho et al., 1989). The airborne transmission of virus particles and high force involved in the act of vomiting increases the area of potential contamination and complicates the ability to confine the virus and to define a particular area to clean. Evans et al. (2002) identified inadequate cleaning and disinfection of hard surfaces, carpets and soft furnishings in a shared exit way in a theater as the likely sources in an outbreak that affected more than 300 people over a 5-day period. Investigations in recurring cases found that repeated and thorough cleaning was often necessary to prevent further outbreaks (Ho et al., 1989; Evans et al., 2002; Wu et al., 2005).

What is known about HuNoV survival, persistence, and transfer on and between foods and surfaces?

Contaminated environmental surfaces can play a critical role in both direct and indirect (secondary) transmission of viruses (Hall, Vinjé et al., 2011; Mbithi et al., 1993). Norovirus has been shown to persist on stainless steel for more than 6 weeks, with greater persistence at cold temperatures (Liu et al., 2012).

Data on transfer of virus particles reveals that it readily occurs but its degree may be affected by many factors such as moisture level, numbers of subsequent transfers, and even the lab protocol set up. Escudero et al. (2012) inoculated a variety of solid surfaces and detected only a gradual reduction of norovirus particles by about 2 logs on Formica, stainless steel, and ceramic surfaces for a period of 42 days. Transfer from “donor” surfaces to “recipient” foods was also investigated and was found to be greater to deli turkey meat than to lettuce. Transfer of norovirus from fingers to stainless steel and foods was assessed by Sharps et al. (2012). They found higher transfer rates from fingertips to stainless steel and foods under wet conditions than dry. The same was true when studying transfer from stainless steel to fingertip with wet conditions enhancing transfer rate. Stals et al. (2013) found more efficient transfer of human norovirus from stainless steel and food surfaces to gloves.

Are there any concerns about HuNoV survival on surfaces that have been subject to the minimum cleaning and sanitizing requirements specified in 4-6 and 4-7 of the 2009 FDA Food Code?

The minimum cleaning and sanitizing requirements are likely adequate much of the time. However, if norovirus contamination is suspected, typical chemical sanitizers used in food establishments are not sufficient to inactivate it. Cleaning may wash some virus particles off, but norovirus requires treatment with a disinfectant for its inactivation as has been described.

Recommendations:

- Perform efficacy studies to evaluate the effectiveness of HuNoV inactivation during warewashing.
- The Food Code should have a specific recommendation for wares potentially exposed to infectious norovirus particles (i.e., use and level of a chemical disinfectant, high temperature) in the event of a norovirus outbreak in the vicinity of or in the establishment.
- Conduct studies to validate effectiveness of laundry procedures against norovirus.
- Norovirus particles persist and can be readily transferred to environmental and food preparation surfaces and foods themselves. Verify cleaning, disinfection of surfaces, and effective hand washing for removal or inactivation of HuNoV.

CHAPTER 4: METHODS DEVELOPMENT AND THE ROLE OF SURROGATES (QUESTION 5)

Charge 5: What methods exist for the detection of HuNoVs in foods and environmental samples and what are the limitations of these methods? How should the issue of virus infectivity be approached when using molecular-based assays? What is the potential for a standard method to be developed and used? What is the value of existing and emerging cultivable surrogate viruses for studying methods development, virus persistence and inactivation, sanitation efficacy, etc?

Noroviruses are a significant cause of foodborne disease worldwide. However, development of extraction and detection protocols is difficult due to the complexity of food matrices. A further challenge is the lack of a robust cell culture system for HuNoV, which leads to the need to utilize surrogate viruses which may be inadequate for studying sanitation efficacy, virus persistence, and inactivation.

HuNoV Diagnostics

In the past, identification of enteric viruses in food samples largely depended on whether the agent grew in cell culture. For those types that do, such as enteroviruses and adenoviruses, detection by virus replication in cell culture demonstrated infectivity as well as their presence. The ability to detect viruses by cell culture is a clear advantage when assessing whether foods are a public health risk. Given the current lack of a cell culture system for HuNoV, several methodologies have been used for the detection of the virus including electron microscopy, protein-based assays such as enzyme-immuno assays, and molecular methods. Of these, RT-PCR and real-time RT-PCR assays have been utilized for a number of years and are now considered the standard for detection of noroviruses in particular in the environmental matrices

such as foods. RT-PCR-based detection is advantageous for detection of low copy numbers of viruses. The presence of inhibitors in the food matrix, inefficient RNA extraction, or degradation of HuNoV RNA can lead to false-negative results. However, false positives are a concern in foods and environmental samples due to the sensitivity and non-specificity of the assay. Internal controls for real-time PCR have been developed to determine the presence of inhibitors in a sample and ensure that reaction conditions are optimal.

Although realtime RT-PCR assays for norovirus are well-established, they were developed for human clinical diagnostics where viral genome copy numbers are generally high. There are several challenges when testing for norovirus in food and environmental samples with these assays, including the lack of standardized methods (concentration, extraction, and for detection), specialized equipment, and the need for trained staff to perform these analyses (Koo et al., 2010). In addition to solving these issues, future methods should also include the ability to detect infectious virus in food and the development of a cell culture system for HuNoV, which will be the next major breakthrough in norovirus research with numerous practical applications. Should such methods be developed, a combination of integrated cell culture methods combined with sensitive PCR detection, as has been developed for other enteric viruses, may be the best option to detect infectious norovirus in environmental samples such as food (Reynolds, 2004).

Sampling and Pre-Sampling Considerations

Optimal strategies for collecting, transporting, and preparing test samples are critical for accurate HuNoV detection. At the most basic level, sampling can be characterized as destructive (i.e. excision sampling of shellfish) or non-destructive (i.e. acid rinse of fruits). Both methods have caveats, and the downstream applications must be considered. While theoretically molecular detection methods like RT-PCR can detect as few as one viral genome in the sample, the reality of complex food matrices necessitates that much higher viral numbers be present for successful detection (Lantz et al., 1994). This can be due to the large sample sizes, particulate matter, presence of food components such as fats and carbohydrates, and molecular assay inhibitors that can be present in abundance in food samples and affect microbial or nucleic acid isolation (Lee and Fairchild, 2006; Liu, 2008). Therefore, viral separation and concentration steps are often a necessary prerequisite in order to reduce sample volume and remove some of the matrix while concentrating the virus. However, there are published methods demonstrating detection of HuNoV at concentrations <10 RT-qPCR (reverse transcriptase quantitative polymerase chain reaction) units/gram or less in clinical, food (shellfish) and environmental samples, (De Medici et al., 2004; Wolf et al., 2007) suggesting that low level sensitivity is possible.

Several strategies have been developed to address effective concentration and purification of HuNoV from food samples (Table 3). These strategies can be broadly classified as physical, charge-based, and bioaffinity approaches or more generally grouped as non-specific and specific separation strategies (De Medici et al., 2004; Dwivedi and Jaykus, 2011). Several physical methods have been examined including co-sedimentation with larger particles by manipulating pH and/or ionic conditions to favor virus adsorption to, or elution from, the food matrix followed by relatively low-speed centrifugation after which the virus-containing phase is recovered for further purification. Alternatively, HuNoV can be separated from the food matrix by charge. The HuNoV capsid (outer viral protein) is highly negatively charged at neutral pH, allowing for

separation of the virus from liquefied food or water samples based on electrostatic interactions between the virus capsid and a positively-charged filter. Both electronegative and electropositive filters (crude filtration) have been employed to concentrate HuNoV from foods. However, the use of filters limits the speed of the procedure, is ineffective for processing samples with high particulate matter, and demands relatively large volumes of eluent for primary and secondary viral recovery. Alternatively, bioaffinity approaches that utilize magnetic separation have been developed in which magnetic beads are coated with capsid-binding ligands like histoblood group antigens (HBGAs) or porcine gastric mucin (PGM) that can be used to concentrate HuNoV from food or environmental water samples (Cannon and Vinjé, 2008; Morton et al., 2009; Stevens and Jaykus, 2004; Tian et al., 2008; Tian et al., 2010; Tian et al., 2011; Tian et al., 2012). Positively charged magnetic beads, which combine charge based separation with the advantages of magnetic separation, have been used. Other methods include ultracentrifugation, polyethylene glycol precipitation, organic solvent extraction to remove matrix-associated lipids, and/or enzyme pre-treatment to break down matrix-associated organic matter, particularly complex carbohydrates. In almost all instances, virus extraction is accomplished by combining two or more of these steps in series (Butot et al., 2014; Dancho et al., 2012; Stals et al., 2012).

Irrespective of the method used for the concentration and of HuNoV from food, extraction controls must be included and have similar properties and be readily detected in all protocol designs. Since any food can become contaminated with HuNoV, it is imperative that extraction controls are added to insure the integrity of the entire protocol. In addition, the inclusion of the extraction control can be used to help standardize methods for detection of HuNoV in food samples. Although procedures to concentrate viruses from foods have improved over time, these methods still have deficiencies and limitations that need to be addressed including variable recovery efficiencies, cumbersome usage, the requirement for expensive equipment, and prohibitive costs (Calgua et al., 2013; Ikner and Gerba, 2012). Further studies are needed to define the optimal procedure for matrix-specific HuNoV isolation and development of internationally- adopted validated protocols.

RNA Extraction Methods

An alternative to purification of HuNoV virions is direct extraction of HuNoV RNA from the food matrix. Commercially available kits differ in their ability to extract viral nucleic acids from complex matrices especially matrices known to contain high levels of inhibitors (Brassard et al., 2009; Rutjes et al., 2006). Studies evaluating methods to extract total nucleic acids from ready-to-eat foods including fresh produce and seafood demonstrated that there are significant differences in viral or nucleic acid recovery depending on the method used and the food matrix that is being tested (Baert et al., 2006; Butot et al., 2007). With respect to specific food matrix interference in recovery, studies demonstrating that the addition of food-specific enzymes (pectinases) may aid in viral RNA isolation from specific foods (Butot et al., 2007) may point the way to the development of more robust matrix specific RNA isolation methodologies. Finally, purification of total RNA by phenol/chloroform extraction is possible depending on the food matrix. Since HuNoVs contaminate a wide variety of foods with different physico-chemical characteristics, a major emphasis should be placed on matrix specific sample preparation methods. Although it is impractical to develop sample preparation methods for each different

food, a more appropriate approach would be to develop approaches based around broad categories of food, separating foods with high levels of components that decrease nucleic acid extraction efficiency from foods that have low levels of inhibitors. For example, high fat and protein components negatively impact nucleic acid extraction efficiency suggesting that fruits and vegetables could be separated from fat and protein-rich foods when developing standardized extraction protocols.

The inability to propagate HuNoV, coupled with low levels of contamination in foods, increases the need for the inclusion of extraction controls in all protocols for detection of viruses in foods. Therefore, nucleic acid extraction controls must also be included in all samples to evaluate extraction efficiency. Further, nucleic acid extraction methods should be designed to allow for high throughput such that diagnostic analysis of multiple samples can proceed rapidly. Clearly, the diversity of PCR inhibitors in food necessitates development in this area and suggests that one system will not work with all food types. The use of appropriate extraction and internal amplification controls (IAC) for the detection of HuNoV has become a necessary part of concentration and extraction protocols and RT-PCR assays. Inhibitors present in food matrices decrease the efficiency of amplification and, if not controlled, can result in the reporting of false-negative results. Several viruses are currently being used as extraction controls; feline calicivirus, murine norovirus, mengovirus, SMSV-17, Tulane virus, and FRNA coliphages (Allwood et al., 2004; Baert et al., 2011; da Silva et al., 2007; Hennechart-Collette et al., 2014). Most of these extraction controls can be propagated in house or purchased from ATCC. For IACs, phage RNA is sometimes utilized and kits composed of armored RNA or specific sequences are commercially available.

Detection and Characterization Methods

The broad genetic diversity of HuNoVs makes primer/probe selections and PCR procedures as variable as extraction methods. A few studies indicate that multiplex or nested assays are necessary to provide adequate sensitivity when analyzing food. In addition to the selection of suitable primers, the amplification products must be confirmed, which is typically accomplished by sequence analysis adding to the complexity of the analysis for laboratories with limited resources. More recently, real-time RT-PCR has been shown to be more sensitive, specific and less time consuming than conventional RT-PCR making it the preferred detection method. In these assays, cross reactive primers and probes are directed in the ORF1-ORF2 junction region for HuNoV (Calgua et al., 2013; Ikner et al., 2012). Stals et al. (2009) reported a quantitative multiplex real-time PCR using forward and reverse ORF1 and ORF2 primers and a ORF1 and 2 florescent probe that has subsequently been used for the detection of HuNoV in soft red fruit (Stals et al., 2011). Performing real time RT-PCR, while less time consuming and more sensitive than traditional RT-PCR, can be more expensive. However, the speed of real-time RT-PCR may be a significant asset when investigating an outbreak. Microarrays that simultaneously detect and genotype noroviruses have been reported (Mattison et al., 2011), but the labor intensiveness and prohibitive cost of such methods precludes their routine use. Other molecular methods to detect HuNoV include isothermal amplification methods such as nucleic acid sequence-based amplification (Lamhoujeb et al., 2009) or LoopAMP (Iturriza-Gomara et al., 2008). These isothermal methods are potentially superior to RT-PCR because such methods can withstand higher levels of nucleic acid inhibitors, and are more rapid than RT-PCR. However,

the general requirement for additional primers/probes as compared to RT-PCR may limit the ability of such methods to detect closely related HuNoV strains. All molecular methods used to detect noroviruses will continue to be challenged by the molecular diversity of the virus.

Detection of Infectious versus Non-Infectious Norovirus Particles

A major caveat of molecular-based detection of HuNoV is the inability of methods, for example PCR, to differentiate between infectious and non-infectious virus (Richards, 1999). Unlike ruptured virus particles that leave the viral RNA vulnerable to rapid inactivation by enzymes in the environment, intact virus particles rendered non-infectious due to damage to capsid proteins will contain intact viral RNA despite being unable to initiate an infection and will be detected by RT-PCR. Numerous studies have attempted to define infectious virus by examining the stability of the viral genome or the integrity of the capsid protein with little success (Baert et al., 2011; Knight et al., 2013). However, Dancho et al. (2012) demonstrated that the ability of HuNoV to bind to porcine gastric mucin (PGM) coupled to magnetic beads (PGM-MB) is dependent on an undamaged capsid protein and could be used as a means to differentiate infectious (binds to PGM-MB) and non-infectious (does not bind) virus (Dancho et al., 2012; Tian et al., 2008). Although preliminary, this methodology could be used to assess inactivation methods as well as incorporated into extraction methods to potentially allow for screening of capsid-intact (i.e. infectious) HuNoV in food.

Outbreak Surveillance

Currently, noroviruses are genetically classified into seven genogroups, GI–GVII, with viruses from GI, GII, and GIV responsible for causing disease in humans. Determining the sequence of the virus in both the patient as well as in the implicated food may help identifying the source of an (foodborne) outbreak (see Chapter 2). To determine if certain norovirus types are more often associated with foodborne outbreaks, to determine the emergence of new norovirus strains, a norovirus outbreak surveillance network named CaliciNet was launched in 2009. During the network's first year, epidemiologic and laboratory data of 552 norovirus outbreaks were submitted to CaliciNet, of which 78 (14%) were associated with foodborne transmission (Vega, Barclay et al., 2011). CaliciNet now collaborates with other NoV surveillance networks like Viro-Net and the National Enteric Surveillance Program (NESP) in Canada and the global norovirus network, NoroNet (Siebenga et al., 2009), to better determine global trends. Given the globalization of the food production and international commerce, surveillance of foodborne disease caused by viruses is essential. To further our understanding of circulating HuNoV strains, especially those associated with foodborne outbreaks, whole genomic sequencing of strains should be explored as a more sensitive tool to link different outbreaks or to link a particular food with norovirus illness. The cost for whole genomic sequencing has continued to decrease and should be explored as a means to detect important genetic changes during an outbreak situation that would be missed by focusing on specific regions of the virus. This information should be made publicly available through databases like GenBank to aid in public health efforts to detect emerging HuNoV and provide invaluable information to the food industry.

Human Norovirus Surrogates

Surrogates are viruses that can be cultivated and share genetic, physical, or chemical relatedness to the pathogen they are chosen to represent. They can be used for disinfection and inactivation studies (Allwood et al., 2004; Hirneisen and Kniel, 2013; Hoelzer et al., 2013; Sattar et al., 2011; Stals et al., 2009; Stals et al., 2011), as indicator organisms of fecal contamination (Jurzik et al., 2010), and as extraction controls for molecular diagnostics (Girard et al., 2013; Mattison et al., 2011). A variety of HuNoV surrogate viruses belonging to the family *Caliciviridae* have been investigated including murine norovirus (MNV), feline calicivirus (FCV), and more recently the primate Tulane virus (Farkas, 2008; Tan and Jiang, 2010). FRNA bacteriophages have also been utilized as surrogates for HuNoV. The genetic relationship amongst the *Caliciviridae* family members can be seen in Figure 6. A brief description of several cultivable surrogates is provided in Table 4.

- **Human norovirus (HuNoV).** HuNoV belongs to the *Norovirus* genus within the *Caliciviridae* family. Genetically, noroviruses can be grouped into different genotypes each of which can be further subdivided into genotypes of which GII.4 is causing the majority of the norovirus outbreaks. Despite extensive work on identifying a cell line that is able to support in vitro culture, including the use of intestinal organoids, HuNoV currently remains unculturable, and no small animal model exists.
- **Feline calicivirus (FCV).** FCV belongs to the genus *Vesivirus* of the *Caliciviridae* family. FCV can be grown in cell culture, and there is a reliable animal model. However, unlike HuNoV, FCV is associated with respiratory disease in infected cats. FCV was widely used because it could be propagated in cell culture.
- **Murine norovirus (MNV).** MNV-1 was first described in 2003 and appears to be a common pathogen of laboratory mice, especially immunocompromised animals (Karst et al., 2003). Like HuNoV, MNV is a member of the genus *Norovirus* (Figure 6) and all MNV belong to the genogroup GV. MNV is shed in feces and transmitted via the fecal/oral route, but it is not uniquely associated with diarrhea and in most cases causes asymptomatic disease (Barron et al., 2011). In immunocompromised mice, MNV can induce hepatitis, encephalitis, and meningitis. MNV is the only norovirus that grows efficiently in cell culture and causes cell lysis and cytopathic effect (CPE) during infection of murine dendritic cells or macrophages.
- **Tulane virus.** The most recently discovered HuNoV surrogate is the rhesus monkey calicivirus or Tulane virus (Farkas, 2008; Tan and Jiang, 2010) which has been proposed as belonging to a new *Recovirus* genus (Figure 6). Like HuNoV, Tulane virus binds to human blood group antigens, and preliminary studies suggest that experimentally-infected macaques develop diarrhea, fever, and shed virus in their feces (Sestak et al., 2012). The virus can be cultured in monkey kidney LLCMK-2 cells. Like HuNoV, recoviruses also are a group of genetically diverse viruses.
- **Bacteriophages.** FRNA (F-specific RNA) coliphage have also been used as HuNoV surrogates. These coliphage are members of the family *Leviviridae* and have a single-stranded positive-sense RNA genome. In nature, FRNA coliphages are shed exclusively in feces of humans and animals (Grabow et al., 2001; Havelaar et al., 1993). Like HuNoV, FRNA coliphages are non-enveloped and are similar in size. The fact that they can be easily grown in the laboratory and, produce readily identifiable plaques on appropriate strains of *E. coli* have made them a favorite surrogate for enteric viruses (Flannery et al., 2009; Dore et al., 2000).

To summarize, all of the surrogates have strengths and weaknesses and their use may depend upon the question being addressed. Of concern, numerous studies evaluating inactivation with common disinfectants, thermal inactivation, and long-term persistence of fecal matrix in environmental conditions have used protocols from which key parameters differ among the studies. Thus, a comprehensive comparative study, including all currently available surrogate viruses, is needed to determine which model is the best surrogate for HuNoV. Additionally, access to several of these viruses is extremely limited and requires use under biosafety level-2 conditions. To accelerate research, these viruses and reagents should be made more available to the scientific community and food industry laboratories with the appropriate laboratory conditions and expertise to safely conduct studies. A suggested repository is the National Institutes of Allergy and Infectious Diseases (NIAID) sponsored and ATCC managed BEI Resources (http://www.beiresources.org). Interested laboratories or groups need to complete a one-time application to access BEI reagents, which are then provided free of charge; the requesting party only pays shipping charges. Making the viruses and reagents readily available would support increased understanding of the usefulness of these agents as HuNoV surrogates.

Given the caveats, our current state of knowledge on the usefulness of surrogates, and the cost associated with conducting surrogate studies, it may be time to consider the costs and benefits of conducting more HuNoV clinical trials, especially for inactivation studies as discussed in Richards (2012). The PGM-binding studies described above are promising and could be linked with limited volunteer studies designed to evaluate whether this assay could be used as a standard for determining if HuNoV has been inactivated. A cost benefit analysis may demonstrate that the costs of supporting clinical trials will be minimal compared to the savings associated with even a modest reduction in HuNoV outbreaks and the positive impact on the food industry.

Knowledge Gaps and Recommendations

In spite of tremendous progress, there remain significant gaps in knowledge including:

1) the ability to differentiate infectious from non-infectious HuNoV in food;
2) validated commodity based extraction protocols; and
3) validated universal (domestic and international) detection assays.

The following recommendations are made to fill these gaps:

1) Make HuNoV sequences more broadly accessible to food laboratories. This would aid in an understanding of the circulating genotypes and adaptation of detection protocols when needed due to evolution and drift of the virus.

2) Develop food-specific standardized HuNoV recovery, concentration and detection procedures. These assays should include the use of appropriate extraction and internal amplification controls (IAC) to evaluate nucleic acid extraction and detection efficiency.

3) Create a widely accessible repository of reference materials including RNA, virus, protocols, surrogate viruses, and virus-like particles to aid in the development of such standardized protocols. A suggested repository is the ATCC-managed BEI Resources.

4) Make a real-time tracking mechanism of foodborne outbreaks, including access to genetic information, accessible to the food industry.

5) Provide competency training opportunities for laboratory personnel involved in HuNoV detection in environmental and food samples.

6) Support the development of methods to differentiate infectious versus non-infectious HuNoV from different types of food.

7) Support human feeding trials to validate HuNoV inactivation and disinfection procedures. This would facilitate the ability of food manufacturers to make decisions regarding disposition of product, potential environmental concern, or process validation and verification and would lessen the dependence on surrogates that do not accurately reflect the properties of HuNoV.

Table 3. Comparison of Sample Preparation Methods [a]

Protocols	Advantages	Disadvantages	Matrix
Polyethylene Glycol (PEG) precipitation[b]	Easy to implement, inexpensive	Concentrates all small particles which leads to inhibition of RT-PCR reactions, time consuming	Shellfish, fruit, leafy greens, meats, green onion, sewage, estuarine and drinking water
Ultracentrifugation[b]	Rapid, eliminates inhibitors, concentration of intact virus, high recovery	Expensive/specialized equipment	Shellfish, fruit, leafy greens, meats, green onion, estuarine water, sewage
Ultrafiltration[b]	Rapid, eliminate inhibitors in non-complex matrices, intact virus	Clog filters with complex matrices, low recovery rates reported	Shellfish, fruit, leafy greens, meats, green onion, estuarine water, sewage
Immunoconcentration[b]	High specificity	Specificity (will need to redesign based on immunogenic differences)	Fruit, leafy greens, meats, green onion
Direct RNA extraction	Rapid ,easy to implement	Low recovery efficiency, high limit of detection (LOD), use of	Shellfish, fruit, leafy greens, meats, green onion, estuarine water,

		hazardous chemicals	sewage
Proteinase K treatment	Easy to implement, rapid, inexpensive	Release nucleic acid from capsid, non-intact virus	Shellfish, fruit, leafy greens, meats
Commercial kits	Easy to implement, rapid	Expensive, high LOD, proprietary/trade secret	Shellfish, fruit, leafy greens, meats, green onion, estuarine water, sewage
Cationic separation	Rapid, eliminate inhibitors	High LOD with complex matrices, specialized equipment	Fruit, leafy greens, green onion
Direct wash with buffer	Rapid, easy to implement, inexpensive	Limited food matrix (surface contamination)	Fruit, leafy green

[a]Adapted from Stals et al., (2012)

[b]Produces intact viral particles, which could be beneficial if cell culture becomes available. Can be used in conjunction with RT-qPCR.

Table 4. Characteristics of HuNoV Surrogates[a]

Characteristic	HuNoV	Feline Calicivirus	Murine Virus	Tulane Virus	Bacteriophage
Classification					
Family	*Caliciviridae*				*Leviviridae*
Genus	Norovirus	Vesivirus	Norovirus	Recovirus (proposed)	Levivirus, Allolevivirus
Host	Human	Feline	Murine	Primates	Bacteria
Characteristics					
Target	Enteric	Respiratory	Enteric	Enteric	N/A
Host Receptor	HBGA	JAM-1	Sialic Acid	HBGA	Bacterial F pilus
Symptoms	Diarrhea	No diarrhea	No diarrhea	Diarrhea	N/A
Grows in culture	No	Yes	Yes	Yes	Yes
Widely available	No	Yes	No	No	Yes
Source	Limited	ATCC	Limited	Limited	ATCC

[a]Modified from Greenberg *et al.*, 1981; Mitchell, 2006; Patel *et al*., 2009; Radford *et al*., 2007; Oehmig *et al*., 2003; Wobus *et al.*, 2006; Taube *et al*. 2012; Farkas *et al*. 2010; Farkas *et al*., 2008; Grabow *et al*., 2001; Leclerc *et al*., 2000; ICTVdB, 2006.

Figure 6. (see separate document for figures)
Evolutionary relationships among viruses representing 5 established genera (*Norovirus*, *Sapovirus*, *Vesivirus*, *Lagovirus*, *Nebovirus*) and a proposed new genus *Recovirus* of the family *Caliciviridae*. Noroviruses can be genetically grouped into at least 5 genogroups. (Graphic developed by Drs. Everardo Vega and Jan Vinjé, 2014)

CHAPTER 5. RISK ASSESSMENT (QUESTION 8)

Q 8: What data are available, and what data are still needed, to conduct a formal quantitative microbial risk assessment of HuNoV transmission in high-risk commodities? Please take into account exposure (levels/frequency) and dose response. When addressing exposure, consider the potential (if any), for zoonotic and secondary transmission of HuNoVs.

NACMCF Interpretation of the Term "High-Risk" Commodity in Reference to HuNoV

As noted in Chapter 2, the food vehicles most commonly associated with foodborne HuNoV outbreaks include molluscan shellfish, sandwiches, salads, and fresh produce. In fact, HuNoVs are the most common microbial hazards linked to outbreaks associated with fresh produce (i.e., fresh fruits, vegetables, and leafy green salads). While the Committee acknowledges these foods have been more frequently associated with HuNoV infections, there are inadequate data with which to identify what point(s) in the supply chain is responsible for contamination. Further, the Committee contends that "high-risk" is more a factor of the practices used in production, handling and distribution of the commodities, rather than an inherent property of the commodity. For example, oysters grown and harvested in waters likely to contain HuNoV are clearly a high-risk food, but oysters grown in a controlled aquaculture environment would not have the same level of risk. Similarly, leafy greens that are mechanically harvested and packaged without further human contact before reaching the consumer would pose a different level of risk than leafy greens that are handled numerous times between harvest and consumption.

Microbial Risk Assessment

Risk assessment is one of the components of risk analysis together with risk communication and risk management (WHO/FAO 2006). According to the World Health Organization, "*it is the scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards.*" (WHO/FAO 2006). Risk Assessments inform risk managers of the various options that can be considered for minimizing the risk in question. A planning and scoping step should be considered before initiating any risk assessment (National Research Council, 2009; USDA/FSIS/EPA, 2012; US FDA, 2002). During this stage the questions to consider are - whether a risk assessment is appropriate, feasible, and can actually be performed. These considerations may sometimes prompt risk managers to make decisions without conducting a formal risk assessment. When a decision is made to proceed with a risk assessment, a process that includes the stages listed below must be followed. (Adapted from NRC, 1983; NRC, 2009; Codex, 2013; USDA/FSIS/EPA, 2012)

- **Hazard identification:** The collection of information on the pathogen, food process, risk factors and disease that is relevant to the risk assessment

- **Hazard characterization:** Hazard characterization or dose-response relationship describes the nature and extent of the adverse health effects to individuals from consuming a specified number of the pathogen. It includes consideration of the virulence of the pathogen strain and susceptibility of individual human subgroups to illness.
- **Exposure assessment:** The evaluation of the degree of intake likely to occur. It considers frequency of contamination, determination of the probability of consuming the pathogen, and the cell numbers consumed. It includes initial contamination frequency and pathogen cell numbers, the effect of any dilution, mixing, inactivation processes, the amount of growth during transportation and storage, frequency of recontamination, and handling practices by the food handler or preparer.
- **Risk characterization:** Integration of hazard identification, hazard characterization and exposure assessment into an estimation of the adverse effects likely to occur in a given population, including attendant uncertainties in the data used to estimate risk.

A risk assessment is one of the most objective and systematic ways to inform the management of food safety risks. A risk assessment can be qualitative, semi-quantitative, or quantitative. It can focus on one or several products; it can cover the whole food continuum from farm to fork or focus on a specific sector such as retail (Ruzante et al., 2013). Risk assessments need to be fit for purpose, so it is crucial to have clearly defined risk management questions to plan and define the scope. According to the USDA/EPA microbiological risk assessment guidance, risk assessment goals can include : a) mitigate (e.g. adverse effects or risk from a specific event; b) confirm (e.g., to determine if regulations, polities, standards, criteria, and/or goals are adequate); c) decide whether and/or how to regulate (e.g., as needed to establish regulations, policies, standards, criteria, and/or goals); and/or, d) investigate (e.g., to determine research or other requirements that would enhance predictive and/or risk ranking capabilities, or facilitate completion of a screening or feasibility assessments). These goals would be defined as part of the risk management question (USDA/EPA, 2012). Availability of data, timeframe, financial and personal resources will also influence the scope of the risk assessment; however, these factors are secondary to the risk management question (Ruzante et al., 2013).

Charge 8 did not provide specific risk management questions for the Committee to consider, but below are examples of concerns related to HuNoV that a risk assessment would be able to address:

1. What are the high risk foods for the transmission of HuNoV according to their production, processing and handling practices?

2. What are the different routes of transmission (including non-foodborne routes) and their relative contribution to the burden of HuNoV?

3. What are the roles of retail and restaurant food handlers versus farm workers that would contribute to contamination with norovirus?

4. What measures, such as segregating sick, infected food handlers from handling food and food contact surfaces, are available to control HuNoV contamination in the different steps of

the food continuum? How effective are they in mitigating HuNoV in foods, and reducing subsequent risk of illnesses and deaths?

5. What is the risk from exposure to HuNoV in aerosols? What control measures are effective, especially in close settings? For example, how far from a vomit incident should cleaning and disinfection be performed?

6. What are the risks associated with food production, handling and processing practices during pre-harvest and post-harvest handling of foods?

7. Is product testing an effective means of reducing public health risk of norovirus infection?

8. Is there a public health benefit to establishing microbiological criteria for norovirus in foods?

Whether the risk assessment method is qualitative, semi-quantitative or quantitative, assumptions and approaches ought to be properly documented; uncertainties and variability have to be clearly articulated so assessments are transparent and can be reproduced. This systematic approach provides insights to the process being evaluated, demonstrates its impact on risk assessment options, and identifies data gaps that can help guide future research. Adequate quality and availability of data and appropriate technical skills of the risk assessor(s) are necessary for the development of a good and reliable risk assessment.

Data Availability and Needs for Performing a Quantitative Risk Assessment for HuNoV

There will be different data needs for efforts focusing on a specific commodity or that try to identify high risk foods or production practices. However, the table below tries to capture the main data needs and to reach a qualitative judgment regarding the adequacy of existing data under each of the four components of a microbial risk assessment. The Committee ranked the adequacy of the data according to the scale 0 to 2 where 0 = no information; 1 = some information; 2 = sufficient information (adequacy of data includes availability and quantity).

Data Needed	Details	Adequacy of Data	Reference
	Hazard Identification		
HuNoV characteristics	*Caliciviridae*, small (27-40nm), round structured, non-enveloped, positive sense, single strand RNA virus, 7.6 to 7.7 kb.	2	Green, 2013
What are the clinical manifestations of HuNoV infection?	Typically self-limiting disease ranging from asymptomatic to acute onset nausea, vomiting, diarrhea and abdominal pain. Typically lasts for 12-60 hrs. However, more severe symptoms and fatalities have been reported.	2	Green, 2013
What is the availability of	Most common detection method is real time and standard RT-PCR assays	2 (patients) 1 (food)	Green, 2013

Data Needed	Details	Adequacy of Data	Reference
detection methods for patients and food?	although enzyme immunoassays (EIA) are available for patient samples.		
How frequent is the disease?	Very common.	2	Chapter 2
Exposure Assessment			
Is illness seasonal?	Person-to-person is primarily associated with the winter season. Much less so for foodborne infections, except for shellfish-associated infections.	2	Chapter 2; Wikswo and Hall, 2012; Achmed,2013
What proportion of HuNoV infections are foodborne versus person-to-person?	It is estimated that that 16-26% are foodborne; however, more information is needed for better accuracy.	1	Chapter 2; Vega, Barclay et al., 2014; Hall et al., 2014
Does food get contaminated via the environment?	Some food contamination is thought to come from water contaminated with human feces or sewage-contaminated irrigation water. However, data are lacking on some food products, e.g., produce. Contamination by fomites is possible.	2 for shellfish 1 for produce	Chapter 2
How persistent is norovirus in the environment (e.g., soil, water)	May persist for a long time, but hard data are missing.	1	Green, 2013
Is food contaminated via human contact?	Yes, by direct fecal-oral transmission or indirectly such as by aerosolization of vomitus.	2	Hall, Vinjé et al., 2011
What is the incidence of HuNoV infections in food workers? What proportion is asymptomatic?	Estimated to be the same as in general population, where studies suggest up to 10% of the population are ill each year. Perhaps 1/3 of infections are asymptomatic.	1	Chapter 3
Are animals potential sources of	More work is needed to determine if there is an animal reservoir for HuNoV. HuNoV has been isolated from several	0	Summa, M., et al., 2012; Mesquita et

Data Needed	Details	Adequacy of Data	Reference
contamination? What is the prevalence (and level) of HuNoV in animals	animal species, but the level and prevalence is unknown.		al. 2010; Mesquita et al., 2013
Which foods are the sources of illnesses?	Raw and RTE foods are more often epidemiologically linked to outbreaks; the food is often just a vehicle of contamination, rather than origin. With the exception of shellfish, the specific food item is less important than how it was prepared.	2 for outbreaks 0 for sporadic	Chapter 2; Painter et al., 2013
What is the frequency and level of norovirus contamination in food?	Better assays and more studies are needed. The frequency and level may vary depending on the region of origin, the type of food, and handling and preparation of the food product.	1	Woods and Burkhart, 2010; Lowther et al., 2012; DePaola et al., 2010; Mattison et al., 2010; Baert et al., 2011
What control measures are available and how effective are they (e.g., what is the efficacy of sanitation, hand washing)?	Clean and disinfect environmental surfaces with an appropriate product (e.g. 10% solution of household bleach). Prevent fecal contamination of food and subsequent ingestion. If drinking or recreational water is suspected, treat with high-level chlorination. Inactivated by heat treatment, UV radiation, and ozone treatment but log reduction usually not quantified due to method limitations (lack of appropriate surrogate, difficulty in quantifying infectious virus particles). Some studies showed that pasteurization of solid foods at 70ºC for 2 min., or HTST processing of milk/ ice cream mix at 71.7ºC may not be adequate to inactivate HuNoV.	1	Chapter 3

Data Needed	Details	Adequacy of Data	Reference
What are the virus transfer rates from hands, water, vomit and fomites to product?	Estimated to transfer readily. There are some direct transfer studies, epidemiological data and surrogate studies, but more information is needed.	1	Mokhtari and Jaykus, 2009
What are the levels of virus shed in the feces and vomitus?	Can be shed at high levels in the stool (10^{11}/gram) and vomitus (10^{4}/gram).	2	Chapters 2 and 3
What are the consumption rates of the different commodities associated with norovirus infection?	NHANES, ERS disappearance data, FoodNet population survey, and commodity group data.	2	CDC, 2014 NHANES; USDA ERS Food Avail Doc.; CDC, 2014 FoodNet
Hazard Characterization			
What are the subpopulations of interest?	All people may be predisposed but young children, older adults and immunocompromised individuals are considered high risk groups.	1	Chapter 2
What are the frequencies of outcomes (e.g., incidence, hospitalization and mortality rates)?	Estimated annual number due to norovirus in the U.S. of illnesses: 19 – 21 million hospitalizations: 56,000 – 71,000; deaths: 570-800.	2	Chapter 2
What is the infective dose?	Estimated 18-1000 viral particles; may vary by strain, based on modeling data from two human feeding trials.	1	Chapter 2
What are the factors influencing the infectious dose of HuNoV?	Non-secretors (20% of Caucasian population) are not susceptible and therefore no infectious dose can be determined. Previous infection confers strain-specific immunity, though duration is unclear. Otherwise, little information available.	0	Chapter 2
Is severity related to strain	Infections with GII.4 strains are more severe than are those due to other	1	Chapter 2

Data Needed	Details	Adequacy of Data	Reference
variations?	genotypes. More data are needed.		Desai et al., 2012
What is the attack rate from outbreaks?	Attack rate is about 50% due to genetic resistance against a particular strain.	2	Noda et al., 2008

Recommendations and Gaps in Risk Assessment

While risk assessment models and studies have been undertaken and are in progress, significant data gaps exist that generate large uncertainties and variability in any quantitative microbial risk assessment of HuNoV transmission in high-risk commodities to be used for policy decisions today. It is important to note that risk assessment efforts have, and will continue to be, strongly hindered by the lack of tissue culture (*in vitro* replication) or practical small animal (*in vivo*) models for propagation of human norovirus. Current molecular detection methods (i.e. RT-PCR) cannot distinguish virus particles that are potential threats to public health from inactivated virions since inactivated norovirus capsids often remain largely intact which protects viral RNA from degradation. While receptor-like binding assays (i.e. PGM) can now provide some inferred estimate of HuNoV infectivity, these methods have limitations. Moreover, how applicable these will be to food matrices is unknown. Thus successful quantitative risk assessment and analysis awaits future development of an *in vitro* HuNoV propagation method. Insufficiencies in norovirus active surveillance and reliable surrogates also inhibit conclusions on norovirus prevalence, significant routes of transfer and in validations and effectiveness protocols for norovirus mitigations. These insufficiencies in turn, inhibit quantitative risk assessments. The Committee stresses the importance of using risk assessment as the basis for developing science-based policies and mitigation strategies, and recommends that Agencies develop clear risk management questions that will guide research efforts for data collection for future quantitative risk assessments.

Appendix A in the Appendices of this document is a summary of published norovirus risk models and related references.

Chapter 6: PREVENTION AND CONTROL (QUESTION 9)

Charge 9: Do certain types of facilities, such as schools, long-term care facilities, restaurants, cruise ships, airplanes, or carriers of foods in interstate conveyance, require a specific HuNoV control strategy? If this need exists, the Committee should develop a generic plan by which to control HuNoV transmission in restaurants and institutions that could be used as a template upon which facility-specific plans could be based.

The Committee concluded that a generic plan could not be developed at this time due to the existing gaps in the data related to transmission and secondary transmission of the illness, methodology, infectivity, sanitation and control of HuNoV. Instead, the Committee has made recommendations based on several currently available guidelines on norovirus prevention,

control, and outbreak management. The Committee acknowledges that certain sectors of the industry have developed protocols to control and respond to HuNoV transmission (e.g. Cruise Ship Industry, U.S. Navy, and FMI). In 2011, the CDC developed and published norovirus outbreak management and disease prevention guidelines (Hall, Vinjé et al., 2011). While these general principles were designed to be applicable across all outbreak settings, each facility should develop and implement a risk reduction plan with different levels of preventive measures/controls designed based on risk. The example below outlines the risk and associated actions in a Level 1, 2, 3, Green, Yellow, and Red format based on low, moderate, and high risk (Petran, 2014; FMI, 2010).

Level 1 - GREEN (low risk):

- Standard procedures – maintain hygiene when norovirus poses no direct threat.
- Follow standard procedures for washing, rinsing and sanitizing hard surface food contact surfaces.

Level 2 YELLOW (moderate risk):

- Risk reduction – a heightened defensive response to an outbreak in your area, such as a nearby restaurant, foodservice/industry, long-term care facility or community at large.
- Additional Considerations (in addition to performing standard procedures for Level 1/Green/low risk):
    - Wash and sanitize food contact surfaces and employee hands on a frequent basis.
    - Change out utensils on buffet line on a more frequent basis.
    - Monitor employee health closely.
    - Perform training, reinforcing cleaning and sanitizing.
    - Reinforce all personnel hygiene requirements with particular attention to hand hygiene.
    - Clean and disinfect “high-touch” hard surfaces on a more frequent basis (3 times or more daily depending on usage): equipment handles, sinks, door handles, paper towel dispensers, soap dispensers, handrails, drinking fountains, toilets, urinals, in-use utensils, phones, intercoms, computers and keyboards, etc.
    - Special attention, including increasing the frequency and rigor of sanitation, should be given to restrooms as it is common for sick individuals to use public restrooms following an incident.

Level 3 RED (high risk):

- Remediation –a focused response to an outbreak in your facility, designed to break he chain of infection or illness such as when there is a suspected or confirmed norovirus incident(s) identified within the facility.
- In addition to the performing the activities listed under Levels 1 (Green) and 2 (Yellow), remediation is required.
- Incident Remediation
    - An immediate assessment and restriction to the area of contamination and the area to be disinfected.
    - Careful treatment and removal of the contaminant for the protection of human health and the environment.

- Cleaning and disinfection of the area and surfaces, as described above, after an incident of vomitus or stool contamination.
- Bag, seal and discard all disposable cleaning equipment; disinfect any tools or other non-disposable items used in the clean-up.
- Thoroughly wash face and hands using defined procedures and soap.
- Open the affected area following natural drying.

Facilities should have a written plan in place that includes standard operating procedures and procedures to prevent an occurrence, and promote risk reduction, remediation and incident clean-up. The facility should have the appropriate bodily fluid spill kits, cleaning chemicals/disinfectants and tools on hand in case of an incident. Specific standard operating procedures should be developed which are applicable to particular job activities (e.g. food handlers, patient-care, and child-care workers, cleaning and sanitation associates) and for certain types of environmental conditions including closed settings such as cruise ships, schools, hospitals, and long-term care facilities and for more open venues such as restaurants. Additional emphasis should be placed on prevention and hygiene in circumstances where employees are multitasking and working between patient care and/or child care and food handling. Procedures should be reviewed and verified on a regularly scheduled, pre-determined basis to ensure the procedures are still effective and employees are properly trained. Having proper procedures and tools in place prior to a HuNoV incident will help a facility reduce the risk and recover, should an incident or outbreak occur.

CHAPTER 7. RECOMMENDATIONS

1) Restrict bare hand contact for RTE foods. Contaminated hands are key transmission vehicles for foodborne norovirus. While hand washing is clearly important, it is not clear that thorough hand washing is sufficient to prevent norovirus contamination of uncooked foods. Also due to the resilience of HuNoVs, current hand sanitizers, based on inactivation of surrogate viruses, are of dubious value for inactivation of HuNoV hand contamination, when used alone or with hand washing. Therefore, food-handling establishments should prohibit bare-hand contact with RTE foods whenever practical.

2) Consider when validating inactivation and other protocols for HuNov that surrogates, though currently necessary, are of limited value. Current surrogate viruses (i.e. MNV, FCV, Tulane virus) are not adequate representatives of HuNoV properties. Funding and research efforts should be directed toward identifying or developing *in vitro* replication systems, small animal models, and infectivity assays for HuNoVs. Although expensive and logistically-complex, human volunteer studies are currently the only direct means of assessing HuNoV inactivation and vaccine efficacy. Enhanced research funding of these studies should be supported. While current surrogates may be of limited value, surrogates need to be developed for in-plant validation and verification of mitigation and control strategies.

3) Emphasize molecular biological evaluation of HuNoV: Advances in DNA sequencing technology and metagenomics now make it possible to generate complete HuNoV strain sequence information. More complete strain sequence information will aid in outbreak tracking and food trace-back efforts, assessment of geographic strain circulation, and

dynamics of strain evolution and transmission. HuNoV reporting databases (e.g. CaliciNet, Vironet) should not only be better coordinated and made more user-friendly but should provide complete, timely, readily-accessible sequence information to appropriate public health authorities.

4) Develop methods to cultivate HuNoV: The primary barrier to advancing the understanding of HuNoV transmission, ecology, epidemiology, control and detection is the lack of a cell culture method. This would reduce the current dependence on surrogates that are woefully inadequate. In addition, the availability of a cell culture method would allow for the development of detection assays that distinguish between infectious and non-infectious virus.

5) Create rapid methods for clinical and food applications: A rapid, accurate, inexpensive method for clinical use to diagnose HuNoV infections is needed. This will facilitate detection and reduce underreporting thus providing better estimates in burden of illnesses. Similarly, such methods should be developed or adapted for HuNoV testing of different food matrices.

6) Validation of Food Code health, hygiene, and handling practices for control of HuNoV: The major risk factors for food service establishments contributing to foodborne illness are improper holding temperatures, inadequate cooking, contaminated equipment, and poor personal hygiene. These have been validated for bacterial pathogen control but have not been for HuNoV.

7) Accessible repository of research materials: Create a widely accessible repository of reference materials including RNA, virus, protocols, surrogate viruses, virus-like particles, etc., to aid in the development of such standardized protocols. A suggested repository is the ATCC-managed BEI Resources.

DEDICATION

The NACMCF and the editors of the *Journal of Food Protection* dedicate this article to Dr. Dean O. Cliver, who was to originally serve on the committee, but passed away on May 16, 2011. Dean's preeminence as a virologist and food and public health microbiologist is greatly missed.

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Chapter 8. REFERENCES (see separate document)

Chapter 9. APPENDICES

9.1. Glossary of Terms

CaliciNet - CaliciNet is a national norovirus outbreak surveillance network of federal, state, and local public health laboratories in the United States, coordinated by the CDC. The CDC launched CaliciNet in 2009 to collect information on norovirus strains associated with gastroenteritis outbreaks in the United States.

Class I recall - A situation in which there is a reasonable probability that the use of or exposure to a violative product will cause serious adverse health consequences or death.

Class II recall- A situation in which use of or exposure to a violative product may cause temporary or medically reversible adverse health consequences or where the probability of serious adverse health consequences is remote.

Class III recall - A situation in which use of or exposure to a violative product is not likely to cause adverse health consequences.

Council to Improve Foodborne Outbreak Response (CIFOR): A multidisciplinary working group convened to increase collaboration across the country and across relevant areas of expertise in order to reduce the burden of foodborne illness in the United States.

Disinfectant - A substance, or mixture of substances, that destroys or irreversibly inactivates bacteria, fungi and viruses, but not necessarily bacterial spores, in the inanimate environment. EPA registers three types of disinfectants based on the type of efficacy data submitted: Limited, General (or Broad-spectrum), and Hospital. (http://www2.epa.gov/pesticide-registration/pesticide-registration-manual-chapter-4-additional-considerations)

Food Employee – An individual working with unpackaged food, food equipment or utensils, or food-contact surfaces. For the purposes of this document, the terms food worker", "food employee", "food handler", "food preparer" and "restaurant worker" should be considered synonymous.

GenBank - The GenBank sequence database, produced at National Center for Biotechnology Information (NCBI), is an open access, annotated collection of all publicly available nucleotide sequences and their protein translations.

Genogroups GI-GVII - Noroviruses are divided into seven genogroups (GI–GVII). GI, GII and GIV are known to infect humans.

Genotyping – Testing to determine the complete genetic constitution of an organism or group, as determined by the specific combination and location of the genes on the chromosomes.

ID_{50} – The median infectious dose. The term is used to designate the dose of an infectious organism required to produce infection in 50 percent of the experimental subjects.

Isothermal amplification methods – Also referred to as sequence-specific isothermal amplification protocols, these sequence-specific DNA amplification methods, in contrast to PCR, often do not require changing the reaction temperature. The methods are extremely fast and do not require thermocyclers.

Microarray - A supporting material (as a glass or plastic slide) onto which numerous molecules or fragments usually of DNA or protein are attached in a regular pattern for use in biochemical or genetic analysis

Outbreak – An acute appearance of a cluster of an illness that occurs in numbers in excess of what is expected for that time and place. In the case of a foodborne outbreak, the source is often a specific food vehicle that contain on specific outbreak clone.

Preharvest - Refers to activities on the farm or ranch that occur before crop or livestock products are sold.

Postharvest - Refers to activities that occur immediately following harvest in crop production and immediately after slaughter in animal production.

Risk Assessment - The scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards.

Sanitizer - A substance, or mixture of substances, that reduces the bacterial population in the inanimate environment by significant numbers, (e.g., $3\log_{10}$ reduction) or more, but does not destroy or eliminate all bacteria. (http://www2.epa.gov/pesticide-registration/pesticide-registration-manual-chapter-4-additional-considerations)

Surrogate – The term surrogate is used to indicate a substitute for an item of interest. In microbiology, surrogates are selected from a group of well characterized organisms and have the following desirable attributes: nonpathogenic; inactivation characteristics and kinetics that can be used to predict those of the target pathogen; behavior similar to the target pathogen when exposed to formulation and/or processing parameters (for example, pH stability, temperature sensitivity, and oxygen tolerance); stable and consistent growth characteristics; easily prepared to yield high-density populations; once prepared, population remains stable until utilized; easily enumerated using rapid, sensitive, and inexpensive detection systems. (U.S. FDA, 2001)

9.2. List of Acronyms

AGE: Acute Gastroenteritis

ATCC: American Type Culture Collection

BEI: Biodefense Emerging Infections Research Resources Repository

CDC: Centers for Disease Control and Prevention, HHS

CFR: Code of Federal Regulations

CFR: Case Fatality Ratio

CFSAN: Center for Food Safety and Applied Nutrition

cGMPs: Current Good Manufacturing Practices

CODEX: Codex Alimentarius Commission is an international food standards setting body (http://www.codexalimentarius.org/)

CPE: Cytopathic Effect

CSPI: Center for Science in the Public Interest

CVM: Center for Veterinary Medicine

DoDVSA: Department of Defense Veterinary Service Activity

EFSA: European Food Safety Authority

EPA: United States Environmental Protection Agency

EPIA: Egg Products Inspection Act

EU: European Union

FAO: Food and Agricultural Organization of the United Nations

FCV: Feline Calicivirus

FCV-F9: Feline Calicivirus, strain F9

FDA: Food and Drug Administration, HHS

FDOSS: Foodborne Disease Outbreak Surveillance System

FFDCA: Federal Food Drug and Cosmetic Act, FDA

FMI: Food Marketing Institute

FMIA: Federal Meat Inspection Act

FNS: Food and Nutrition Service

FOODNet: Foodborne Diseases Active Surveillance Network

FRNA: F-Pilus RNA Phage

FSIS: Food Safety and Inspection Service, USDA

GAPs: Good Agricultural Practices

GII.4: Genotype 2, clade 4 (HuNoV)

GMPs: Good Manufacturing Practices

GP General Practitioner

GPs: General Practioners

HACCP: Hazard Analysis Critical Control Points

HAV: Hepatitis A virus

HBGA Histo-blood group antigens

HHS: Department of Health and Human Services

HPP: High Pressure Processing

HTST – High Temperature Short Time Pasteurization

HuNoV: Human Norovirus

HuNoVs: Human Noroviruses

IACs: Internal Amplification Control

ICTV: International Committee on the Taxonomy of Viruses

ISO: International Organization for Standardization

LLC-MK2: Monkey Kidney Cell Line (from ATCC)

LoopAMP (LAMP): Loop-mediated isothermal amplification

MMWR: Mortality and Morbidity Weekly Report

MNV: Murine Norovirus

MNV-1: Murine Norovirus strain 1

MOU: Memorandum of Understanding

NACMCF: National Advisory Committee on Microbiological Criteria for Foods

NESP: Canadian National Enteric Surveillance Program

NIAID: National Institute of Allergy and Infectious Disease

NIH: National Institutes of Health

NMFS: National Marine Fisheries Service

NOAA: National Oceanic and Atmospheric Administration

NORS: National Outbreak Reporting System

NoroNet: Global Norovirus Surveillance Network

Norwalk agent previously used to refer to Norovirus

NRA: National Restaurant Association

NRTE: Not-ready-to-eat

NSIL: National Seafood Inspection Laboratory

NSSP: National Shellfish Sanitation Program

NVEAIS: National Voluntary Environmental Assessment Information System

ORA: Office of Regulatory Affairs

ORF: Open Reading Frame

PCR: Polymerase Chain Reaction

PEG: Polyethylene Glycol

PGM: Porcine Gastric Mucin

PGM-MB: Porcine Gastric Mucin conjugated to Magnetic Beads

PPIA: Poultry Products Inspection Act

QMRA: Quantitative Microbial Risk Assessment

QRA: Quantitative Risk Assessment

RNA: Ribonucleic Acid

RTE: Ready-to-eat

RT-PCR: Reverse transcription-Polymerase Chain Reaction

SIP: Seafood Inspection Program

SMSV-17: San Miguel Sea Lion Virus strain 17

USDA: United States Department of Agriculture

VSP: Vessel Sanitation Program

ViroNet: Canadian Norovirus Surveillance Network

WDOSS: Waterborne Disease Outbreak Surveillance System

WHO: World Health Organization

Additional Tables & Figures – (see separate document for Figures).

APPENDIX A. Summary of Norovirus Risk Models and Related Publications 2001-2011

Reference	*Topic Area*	*Comments*
Risk Profiles, Qualitative Risk Assessments, Scientific Opinion, Literature Reviews		
Bassett and McClure, 2008	Qualitative risk assessment for microbiological hazards in fresh fruit and evaluates potential risk management options to control risks.	Based on criteria used, norovirus considered a significant public health burden and hazard in fresh fruit.
Bouwknegt et al., 2013	Risk profile. Norovirus focuses on fresh produce.	Extensive literature review and identified data gaps.
Codex, 2006	Risk profile; norovirus in bivalve molluscan shellfish.	Recommended risk management activities for CCFH to undertake.
De Roda Husman, 2010	Literature review of qualitative and quantitative risk assessment for viruses.	This is a power point presentation; lists key data gaps; puts risk assessment into a broader context.
Drechsel et al., 2010	Review paper; norovirus in wastewater.	See Chapter 4 for a discussion of types of dose-response studies; See Chapter 5 for estimates of norovirus infection risks.
EFSA, 2011	Review of biology, epidemiology, diagnosis and public health importance of norovirus (and other viruses); identifies data needs to support a risk assessment.	Identifies control options and their anticipated impact to prevent or reduce foodborne infections. Recommends that to quantify the efficacy of specific control options, a quantitative risk assessment is needed and should be done for specific priority virus-commodity combinations, including consideration of the target population.
Fanaselle et al., 2010	Draft risk profile (FDA).	Presentation on draft risk profile; risks associated with transmission and control options.
FAO/WHO, 2008	Scientific advice to support risk management activities.	Includes information on available data/ feasibility for risk assessment.
Greening, 2009	Risk profile focused on shellfish; includes data gaps, control measures.	Update of previous risk profile (Greening, 2003).
Lawrence et al., 2004.	Qualitative risk assessment framework of known routes of infection and associated flows of risk. Quantitative transmission models (person-to-person; vegetable/fruit, shellfish).	Identified data gaps (UK) – see page 30, prioritized data needs.

Lund and Lindqvist, 2004	Risk profile (Sweden).	Included knowledge gaps.
University of Liverpool, 2014	Assessing the contribution made by the food chain to the burden of UK-acquired norovirus infection.	Study duration: January 2014 to May 2017. Updates available at: http://www.food.gov.uk/science/research/foodborneillness/b14programme/b14projlist/fs101040#toc-4
USDA, NIFA, 2011	Quantitative Risk Assessment on Norovirus foodborne illness from Ready-to-Eat food.	Under development. Lead on Risk Assessment is LeeAnn Jaykus. Updates available at: www.Norocore.ncsu.edu
Yezli and Otter, 2011	Review article on minimum infective dose of respiratory and enteric viruses.	See section on norovirus.
Risk Ranking, Risk Prioritization		
Anderson et al., 2011	Semi-quantitative attribution risk ranking; useful for prioritization among produce.	Tool available at www.foodrisk.org ; norovirus not in top 10 pathogen/produce pairs.
Batz et al., 2011; Batz et al., 2012	Semi-quantitative attribution risk ranking; useful for prioritization among a variety of foods.	Norovirus ranks 5th for annual disease burden; norovirus/complex food pair ranks 6th.
Newsome et al., 2009	Prototype framework risk ranking tool.	The prototype has been operationalized by FDA, undergone an external peer review, and is available at: https://irisk.foodrisk.org/
Quantitative Risk Assessment Models		
Barker et al., 2013	QMRA model	Estimated risks associated with consuming vegetables irrigated with treated wastewater (greywater).
US FDA, 2011	A collaboration between the U.S. Food and Drug Administration (FDA) Health Canada, the Canadian Food Inspection Agency, Environment Canada, and Fisheries and Oceans Canada, to conduct a quantitative risk assessment on norovirus in bivalve molluscan shellfish.	The risk assessment will focus on contamination of oysters, clams and mussels during growth, harvest, and post-harvest processing and will examine the impact of preventive practices and controls on the level of risk. FDA, on behalf of the collaborative team, requested comments, scientific data and information that would assist in the development of the risk assessment. The deadline for electronic or written submission of comments, scientific data, or information closed on January 18,

		2012. For more information, please consult: Federal Register Docket No. FDA-2011-N-0731
Hamilton et al., 2006	QMRA; estimate annual risk of enteric virus infection, raw vegetables contaminated via overhead irrigation with reclaimed water.	@Risk software; rotovirus used to represent enteric virus group for modeling the dose-response relationship (β-Binomial).
Mahajan, 2009	QMRA for norovirus in drinking water.	Dynamic model of sewer overflow; simulations show a high degree of variability in pathogen loads that would be difficult to measure in a typical sampling program; MS thesis.
Mara and Sleigh, 2010a; Mara and Sleigh, 2010b	QMRA, risk to farmer and consumers from wastewater irrigated crops.	Determine tolerable disease and infection; DALY loss of $9x10^{-4}$; model available at: www.personal.leeds.ac.uk/~cen6ddm/QMRA.html
Masago et al., 2006	QRA; estimate disease burden from NoV in tap water (Japan).	Exponential dose-response model used.
Michaels et al., 2004	QRA; food worker behavior/transmission modeled using Goldsim; interventions tested.	Hand hygiene (HH) compliance and reducing the number of cross-contamination events determined more important than improvements in washing efficacy; HH frequency increases are effective in reducing cross-contamination.
Mokhtari & Jaykus, 2009	Quantitative exposure model; transmission during retail food preparation.	Discussion of data and model limitations.
Petterson and Ashbolt, 2001; Petterson, Ashbolt and Sharma, 2001	Risks from wastewater irrigation of leafy greens.	Model sensitive to virus decay rate.
Schijven et al., 2013	QMRA	Risk associated with climate change. The impact on drinking water, bathing water and oysters were examined for norovirus.
Dose-response Models and Related Outbreak Information		
Baker et al., 2011	Outbreak investigation; useful for dose-response information.	Norovirus in oysters: genogroup I RNA <100 copies/g and Genogroup II RNA at 1,736 copies/g. Serving size = 6 oysters.
Noda et al., 2008	Determination of attack rate (outbreaks); useful for dose-	Comparison attack rates from oyster-related and food-handler outbreaks.

	response.	
Pujol et al., 2009	Cumulative dose-response model.	Dynamic mechanistic model; Rotovirus; examine implications of dose-timing effects for infection transmission system modeling; more data needed to test this approach.
Seitz et al., 2011	Dose-response study.	Long term infectivity of human norovirus in water; 13 subjects challenged with spiked groundwater. Norovirus remained infectious for at least 61 days.
Teunis et al., 2008	Dose-response study.	Estimated ID_{50} as low as 18 virions.
Additional Models & Tools		
Ball et al., 2010	Stochastic epidemic model, incorporating household contacts; useful for exposure assessment.	Starts with a single infectious individual; models spread from individuals and terminates when there is no infective remaining in the population.
Brooks et al., 2005a; Brooks et al., 2005b	Empirically-derived transport model, predict downwind concentrations during land application of liquid biosolids; useful for exposure assessment. models (on farm).	Brooks et al. (2005b) reported norovirus detected 5m or less downwind from application sites; infectious norovirus may be aerosolized.
Noakes and Sleigh, 2009	Mathematical model.	Airborne infection disease transmission model (Well-Riley model).
Zelner et al., 2010	Household transmission dynamics; infection time series.	Model provides an estimate of person-to-person infection rate and average infectious period.