Guidance for Industry and Food and Drug Administration Staff

Class II Special Controls Guidance Document: Norovirus Serological Reagents

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Food and Drug Administration
Center for Devices and Radiological Health
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Division of Microbiology Devices

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Preface

Public Comment

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Class II Special Controls Guidance Document: Norovirus Serological Reagents

1. Introduction

This special controls guidance document was developed to support the classification into class II (special controls) of norovirus serological reagents. These devices detect norovirus antigens in human fecal specimens as an aid in the diagnosis of norovirus infection in the setting of an individual patient with symptoms of acute gastroenteritis when the individual patient is epidemiologically linked to other patients with symptoms of acute gastroenteritis and/or aid in the identification of norovirus as the etiology of an outbreak of acute gastroenteritis in the setting of epidemiologically linked patients with symptoms of acute gastroenteritis. An ELISA-based assay for the detection of norovirus antigens is a qualitative enzyme immunoassay that detects the presence of Norovirus antigens in human fecal specimens. This document does not address norovirus nucleic acid amplification assays, but concepts described here may be helpful in developing nucleic acid based Norovirus detection devices. Please contact the Division of Microbiology Devices in the Office of *In Vitro* Diagnostic Device Evaluation and Safety for further information on norovirus nucleic acid amplification assay submissions.

Designation of a guidance document as a special control means that any firm currently marketing, or intending to market, norovirus serological reagents will need to address the issues covered in this special controls guidance. The firm will need to show that its device addresses the issues of safety and effectiveness identified in the guidance, either by meeting the recommendations of the guidance or by some other means that provides equivalent assurances of safety and effectiveness.

2. Norovirus - Background

Noroviruses are a major cause of acute gastroenteritis; gastroenteritis due to norovirus can occur sporadically due to an unidentified source or as outbreaks in semi-closed settings such as nursing homes and cruise ships. Infection can be spread person-to-person or through a common source such as contaminated food or water. Infection may be asymptomatic in up to 30% of patients; in symptomatic patients, symptoms of vomiting and diarrhea usually occur between 1 - 2 days after exposure and last between 2 - 3 days. Disease manifestations may differ between children and adults, with vomiting more prominent in children. Infection may be life-threatening in patients who become dehydrated and are unable to maintain fluid balance. Virus is shed in stool for an

average of 4 weeks after infection, and prolonged shedding in immunocompromised patients may occur.

Noroviruses (genus Norovirus, family *Caliciviridae*) are a group of related, single stranded RNA, non-enveloped viruses that can cause acute gastroenteritis in humans and other mammals. Five distinct/different norovirus genogroups have been described (GI – GV), but human pathogens have been described only from Genogroup I (approximately 8 genotypes), Genogroup II (approximately 19 genotypes), and Genogroup IV (1 genotype). Genogroup II/genotype 4 virus (GII.4) is the most common cause of acute gastroenteritis outbreaks in the United States; other genotypes are generally less common. Genogroup IV norovirus is a rare cause of disease in the United States. The prevalence of different norovirus genotypes may vary by geographical area as well as change over time in the same area. Noroviruses may also evolve over time due to changes in the VP1 major structural protein.

3. Premarket Notifications - Background

FDA concludes that special controls, when combined with general controls, will be sufficient to provide reasonable assurance of the safety and effectiveness of norovirus serological reagents. Designation of this guidance document as a special control means that a manufacturer who intends to market a device of this type should (1) conform to the general controls of the Federal Food, Drug, and Cosmetic Act (the FD&C Act), including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific issues of safety and effectiveness identified in this guidance document, and (3) obtain a substantial equivalence determination from FDA prior to marketing the device.

This guidance document identifies the classification regulation and associated product codes for norovirus serological reagents (refer to Section 4 - Scope). In addition, other sections of this guidance document list the risks to health and describe measures that, if followed by manufacturers and combined with the general controls, will generally address the risks associated with these devices and lead to a timely premarket notification [510(k)] review and clearance. This document supplements other FDA documents regarding the specific content requirements of a premarket notification submission. You should also refer to 21 CFR 807.87 and CDRH's Device Advice
http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm

Guidance on the content and format for abbreviated and traditional 510(k)s is available at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084365.htm.

4. Scope

The scope of this document is limited to the devices listed below, as described in 21 CFR 866.3395, with the product code OUC. The recommendations contained in this guidance may also assist manufacturers of future Norovirus diagnostic devices that may not fall within these existing classifications in determining how to comply with requirements that apply to their devices, including devices that will be subject to requests for initial classification under section 513(f)(2) of the act ("de novo classification"), as well as subsequent devices that seek determinations of substantial equivalence to future de novo cleared devices. Manufacturers of such devices should contact FDA for clarification about how the recommendations contained in this guidance may apply to their device.

In the companion rule FDA has identified these devices, classified under 21 CFR 866.3395 as follows:

21 CFR 866.3395 – Norovirus serological reagents

(a) Identification. Norovirus serological reagents. Norovirus serological reagents are devices that consist of antigens and antisera used in serological tests to detect the presence of norovirus antigens in fecal samples. These devices aid in the diagnosis of norovirus infection in the setting of an individual patient with symptoms of acute gastroenteritis when the individual patient is epidemiologically linked to other patients with symptoms of acute gastroenteritis and/or aid in the identification of norovirus as the etiology of an outbreak of acute gastroenteritis in the setting of epidemiologically linked patients with symptoms of acute gastroenteritis.

(b) Classification. Class II (special controls). The special control is FDA's guidance document entitled “Class II Special Controls Guidance Document: Norovirus Serological Reagents.” See § 866.1(e) for the availability of this guidance document.

5. Risks to Health

FDA has identified the risks of false negative test and false positive test results, both of which can lead to individual and/or public health consequences, as risks to health associated with this device that require special controls. These risks, and the location of recommendations for addressing them, are summarized in the table below.

Failure of norovirus detection devices to perform as indicated or an error in interpretation of the results may lead to misdiagnosis with significant implications. In the setting of individual patient diagnosis this may lead to improper management of a specific individual; for example, an incorrectly negative test result (false negative result) may lead to inappropriate antibiotic use, or an incorrectly positive test result (false positive result) may lead to a delay in finding the true diagnosis. Perhaps more importantly, failure to identify the cause of a norovirus outbreak due to a falsely negative result has the potential to allow additional spread of the outbreak and/or require unnecessary additional investigation; an incorrectly positive result during an outbreak investigation may lead to the institution of unnecessary patient restrictions and/or substantial

efforts at environmental decontamination, as well as perhaps delaying identification of the true pathogen.

The symptoms of norovirus infection, i.e., nausea, emesis, and diarrhea, overlap with other causes of acute gastroenteritis. In the absence of clear symptoms or signs that separate norovirus infections from other etiologies of gastroenteritis, it is likely that the results of a norovirus diagnostic test would strongly influence ascribing the cause of illness to norovirus infection.

In the table below, FDA has identified the issues generally associated with the use of norovirus serological reagents that require special controls. The measures recommended to mitigate these identified issues are in this guidance document, as shown in the table below, in combination with subsection 21 CFR 866.3395. We recommend that you also conduct a risk analysis, prior to submitting your premarket notification, to identify any other risks specific to your device. The premarket notification should describe the risk analysis method. If you elect to use an alternative approach to address a particular risk identified in this guidance document or have identified risks additional to those in this document, you should provide sufficient detail to support the approach you have used to address that risk.

Identified Risks	Recommended Mitigation Measures
A false positive test result for an individual may lead to a potential delay in needed antibiotic treatment (when appropriate) and possibly less thorough laboratory evaluation for the true cause of illness; in the setting of an outbreak investigation, a false positive result may lead to unnecessary environmental interventions and/or significant patient restrictions.	Section 7 (Performance Characteristics) Section 8 (Labeling)
A false negative test result for an individual may lead to potentially unnecessary treatment for other causes of acute gastroenteritis, including possible antibiotic exposure; in the setting of an outbreak, a false negative result may lead to delay in recognizing the cause of the outbreak and additional spread of norovirus infection.	Section 7 (Performance Characteristics) Section 8 (Labeling)

6. Device Description

In your 510(k) submission, you must identify the legally marketed predicate device as required by 21 CFR 807.92(a)(3). You should also identify the applicable regulation and the product code(s) for your device; it is also strongly recommended that you include a table that outlines the similarities and differences between the predicate device (or another legally marketed device for the same intended use) and your device. We encourage you to reference appropriate peer-reviewed articles that support the use of your device for its intended diagnostic use and the

specific test principles incorporated into the device design. We recommend that you describe each of these device elements in detail.

In addition, we recommend that you include the following descriptive information to adequately characterize your device for the detection of norovirus antigen in human fecal specimens.

a. Intended Use

The intended use should specify the nature of the analyte and the target (e.g., norovirus and specific genogroups/genotypes detected by the device), that use is restricted to fecal specimens, the clinical indication(s) for which the test is to be used, and the specific population(s) for which the test is intended. The intended use should state that the test is qualitative and any specific conditions of use. The intended use should also specifically state as part of the clinical indication whether the test is to be used in the setting of diagnosing individual patients (i.e., sporadic norovirus infection) and/or for diagnosis during outbreak investigations.

In your 510(k), you should clearly describe the following information related to the intended use of your product:

- The identity or other recognized characterization of the norovirus genotypes that your device is designed to detect (i.e., strain reactivity).
- How the device test results will be used to aid in laboratory identification of norovirus in specimens from symptomatic patients.

b. Test Methodology

You should describe in detail the methodology used by your device. This should include describing the following elements as applicable to your device:

- The specific test methodology to be used, e.g., immunoassay or immunochromatographic procedure.
- Specificity of monoclonal antibodies for the norovirus genogroups/genotypes of interest.
- Information regarding the rationale for selection of specific genogroups/genotype targets.
- Limiting factors of the assay, e.g., pipetting, incubation, washing, and mixing.
- Sample types (e.g., fecal specimen), collection and handling methods.
- Reagent components provided or recommended for use, and their function within the system (e.g., solid support, buffers, fluorescent dyes, chemiluminescent reagents, substrates, conjugates, other reagents).
- Instrumentation involved in the use of your device, including the components and their function within the system.
- The computational path from raw data to the reported result (e.g., how raw signals are converted into a value) if appropriate. This would include sufficient software controls for identifying and dealing with obvious problems in the dataset. It would also include adjustment for background and normalization, if applicable.

- Illustrations or photographs of non-standard equipment or methods as appropriate.

When applicable, you should describe design control specifications for your device that address or mitigate risks associated with an immunoassay procedure detecting norovirus, such as the following:

- Minimization of false positives due to contamination.
- Developing or recommending validated methods for antigen protein extraction and purification that yield suitable quality and quantity of norovirus from fecal specimens for use in the test system with your reagents, if appropriate. You should address suitable validated extraction method(s) for the different specimen types that your assay claims in its intended use.
- Optimizing your reagents and test procedure for recommended instruments.

In your 510(k), you should provide performance information that supports the conclusion that your design control specifications have been met. You should also provide information to verify the design of your reagents (e.g., rationale for selection of specific antibodies). See Section 7 – Performance Characteristics.

c. Ancillary Reagents

Ancillary reagents are reagents specified in device labeling as "required but not provided" in order to carry out the assay as indicated in its instructions for use and to achieve the test performance claimed in labeling for the assay. For the purposes of this document, ancillary reagents of concern are those specified according to manufacturer and catalog or product number, or other specific designation, in order for your device to achieve its labeled performance characteristics. For example, if your device labeling specifies the use of a specific brand of reagent (e.g., 'Brand X Extraction Buffer'), and use of any other extraction buffer may alter the performance characteristics of your device from that reported in your labeling, then Brand X Extraction Buffer is an ancillary reagent of concern for the purposes of this document.[1]

By contrast, if your device relies on the use of 95% ethanol and any brand of 95% ethanol will allow your device to achieve the performance characteristics provided in your labeling, then 95% ethanol is not an ancillary reagent of concern for the purposes of this document.

If the instructions for use of your device specify one or more ancillary reagents of concern, you should address how you will ensure that the results of testing with your device and these ancillary reagents, in accordance with your instructions, will be consistent with the performance established in your premarket submission. Your plan may include application of quality systems approaches, product labeling, or other measures.

[1] Even if you establish that one or more alternative ancillary reagents may be used in your assay, each of those named alternatives may still be an ancillary reagent of concern. We recommend you consult with FDA if you are unsure whether this aspect of special controls applies to your device.

In order to address this aspect of the special control, your 510(k) submission should address the elements described below. FDA will evaluate whether your plan will help to mitigate the risks presented by the device to establish its substantial equivalence.

(1) You should include in your 510(k) a risk assessment addressing the use of ancillary reagents, including risks associated with management of reagent quality and variability, risks associated with inconsistency between instructions for use provided directly with the ancillary reagent and those supplied by you with your assay, and any other issues that could present a risk of obtaining incorrect results with your assay.

(2) Using your risk assessment as a basis for applicability, you should describe in your 510(k) how you intend to mitigate risks through implementation of any necessary controls over ancillary reagents. These may include, where applicable:

- User labeling to assure appropriate use of ancillary reagents (see "Labeling" for further discussion).
- Plans for assessing user compliance with labeling instructions regarding ancillary reagents.
- Material specifications for ancillary reagents.
- Identification of reagent lots that will allow appropriate performance of your device.
- Stability testing.
- Complaint handling.
- Corrective and preventive actions.
- Plans for alerting users in the event of an issue involving ancillary reagents that would impact the performance of the assay.
- Any other issues that should be addressed in order to assure safe and effective use of your test in combination with named ancillary reagents, in accordance with your device's instructions for use.

In addition, you should provide testing data to establish that the quality controls you supply or recommend are adequate to detect performance or stability problems with the ancillary reagents.

If you have questions regarding identification, use, or control of ancillary reagents, you should contact FDA for advice.

d. Testing Procedures Using Your Device

In your 510(k) submission, you should provide a detailed description of the principles of operation for your device. We recommend that you specifically describe testing conditions, procedures, and controls designed to provide safeguards for conditions that can cause false positive and false negative results, or that may present a biosafety hazard. These include, but are not limited to:

- Description of, or recommendations for, any external controls and/or internal controls (e.g., sample negative controls and/or internal controls that monitor assay performance).

- Overall design of the testing procedure, including control elements incorporated into the recommended testing procedures.
- Features and additional controls that monitor procedural errors or factors (e.g., degradation of reagents) that adversely affect assay performance and detection.

We recommend that you include a description for all additional procedures, methods, and practices incorporated into your directions for use (See Section 8 - Labeling) that mitigate risks associated with testing for norovirus.

e. Specimen Storage and Shipping Conditions

If you recommend specimen storage conditions and or shipping, you should demonstrate that your device generates equivalent results for the stored specimens at several time points throughout the duration of the recommended storage period and at both ends of your recommended temperature range. If a transport medium is recommended for storage or shipping, you should conduct appropriate studies to demonstrate that the device will perform as described when the specimen is preserved in the transport medium.

f. Interpreting Test Results/Reporting

In your 510(k), you should describe how positive, negative, equivocal (if applicable), or invalid results are determined and how they should be interpreted. In your 510(k) submission, you should indicate the cut-off values for all outputs of the assay.

You should provide the specific cut-off value for defining a negative result of the assay. If the assay has only two possible output results (e.g., positive and negative), this cut-off also defines a positive result of the assay.

If the assay has an equivocal zone, you should provide cut-off values (limits) for the equivocal zone and recommendations for how the user should follow up the equivocal results. If your interpretation of the initial equivocal results involves retesting, your 510(k) should address:

- Whether retesting should be done by the same assay or a different method.
- Whether retesting should be repeated from the same preparation, a new extraction, or a new patient specimen.
- An algorithm for defining a final result by combining the initial equivocal result and the results after retesting if retesting is done by the same assay as the initial testing. (This algorithm should be developed before the pivotal clinical studies that evaluate the clinical performance of the assay.)

If the assay has an invalid result, you should describe how an invalid result is defined. If internal controls are part of the determination of invalid results, you should provide the interpretation of each possible combination of control results for defining the invalid result. You should provide recommendations for how to follow up any invalid result, i.e., whether the result should be reported as invalid or whether retesting is recommended. If retesting is recommended, you should provide information similar to that for retesting of equivocal results

(i.e., whether retesting should be repeated from a new aliquot of the same sample or a new patient specimen).

In addition, you should describe how you monitor results over time to identify changes in performance due to biological changes within the virus genetic lineage, or changes in performance when prevalence changes from the existing prevalence at the time your product is evaluated.

7. Performance Characteristics

a. General Study Recommendations

Your 510(k) submission should include detailed descriptive information regarding the studies that you conducted to establish each of the performance characteristics outlined below.

Prospective clinical studies are recommended to determine the performance of your device in conditions similar to the proposed intended use. In general, for both clinical studies and analytical precision studies, we recommend that you conduct testing at 3 sites that are representative of where you intend to market the device (e.g., clinical laboratory sites).

In order for FDA to accurately interpret acceptance criteria and data summaries contained in your application during our review, we recommend that you provide appropriate specific information in your 510(k) submission describing the protocols used during your assay development. This information is also important to aid users in understanding information in your labeling. When referring to Clinical and Laboratory Standards Institute (CLSI) protocols or guidelines, we recommend that you indicate which specific aspects of the protocols or guidelines were followed.

We recommend that you contact FDA prior to initiating your clinical studies program to obtain feedback regarding your planned studies and the intended uses that are planned for inclusion in your 510(k) submission.

b. Analytical studies

We recommend that you establish the following performance characteristics for your norovirus immunoassay in your 510(k):

(1) Analytical Sensitivity

(a) Limit of Detection

We recommend that you determine the limit of detection (LoD) of your device using serial dilutions of well characterized norovirus positive fecal specimens. LoD should be determined for at least one well characterized Genogroup I and one well characterized Genogroup II sample (preferably GII.4). The testing may consist of analysis of 20% weight/volume (w/v) preparations of the original specimen, and a further 6 log dilutions, all to be carried out in triplicate for each sample.

The limit of detection (LoD) of the device should be estimated as the level of norovirus (expressed as the number of viral particles/gram of fecal sample and RNA copy number/gram of fecal sample) that give a minimum of 2 out of 3 tests positive in your device. The estimated LoD should be confirmed by preparing at least 20 additional replicates at the proposed LoD concentration and demonstrating that the virus can be detected 95% of the time.

To determine the absolute viral particle numbers for the dilutions, electron microscopy (EM) testing of your norovirus positive samples should be performed at the lowest dilution (i.e., the initial dilution) where viral particles numbers can be reliably counted by EM. Absolute viral particle numbers in viral particles/gram of fecal sample for each higher dilution can be extrapolated from the lowest dilution.

To determine the RNA copy numbers for the dilutions, a well characterized and validated real-time reverse transcriptase polymerase chain reaction (RT-PCR) should be used. (See "Reference Assays" in Section 7.f.) RNA copy numbers should be established for each dilution from the real-time RT-PCR testing.

The protocols used for the EM testing and the real-time RT-PCR assay (and the source of the protocols) should be included in your 510(k) submission; these protocols can also be submitted to FDA for review as a pre-IDE submission prior to initiating analytical studies.

(b) Strain reactivity

A panel of well characterized stool samples should be tested by the immunoassay to establish device strain reactivity. The status of this panel should be characterized using bi-directional sequencing of a partial region of the norovirus genome identifying strains at the genogroup and genotype levels.

It is recommended that the following norovirus strains be tested in your strain reactivity study:

Genogroup I: GI.1, GI.2, GI.3, GI.4, GI.5, GI.6, GI.7, GI.8
Genogroup II: GII.1-10, GII.12, GII.13, GII.14, GII.15, GII.16, GI1.17
Genogroup IV: GIV.1

You should cite literature and/or other evidence for norovirus strains excluded from your study. In addition, additional strains may be appropriate for inclusion based on clinical and epidemiological trends at the time the device is being developed.

The protocol for strain reactivity studies should specify the viral inoculum to be used during strain reactivity studies; it is recommended that both a "high positive" inoculum and a concentration near the limit of detection (i.e., "low positive") be tested.

The results of strain reactivity testing, i.e., strains that are or are not detected by the product, should be listed in the product labeling.

(2) Analytical Specificity

(a) Cross-reactivity

We recommend that you test for potential cross-reactivity against the non-norovirus gastrointestinal pathogens listed in Table 1. Cross-reactivity should be tested at medically relevant viral and bacterial levels, usually 10^6 cfu/ml or higher for bacteria and 10^5 pfu/ml or higher for viruses. Viral and bacterial isolates used for cross-reactivity studies should have identity and titer confirmed prior to testing.

Table 1. Microorganisms Recommended for Cross-reactivity Studies.

Test Organism	Type/Strain	Test Organism	Type/Strain
Bacteria:			
Acinetobacter lwoffii		*Salmonella bongori*	
Aeromonas caviae complex		*Salmonella enterica*	
Aeromonas hydrophila complex		*Serratia proteamaculans (liquefaciens)*	
Campylobacter coli		*Shigella flexneri*	
Campylobacter jejuni		*Shigella sonnei*	
Citrobacter freundii		Toxin-producing *Staphylococcus aureus* (food poisoning)	
Clostridium difficile toxin A/B producers		*Streptococcus agalactiae*	
Clostridium sordellii		*Streptococcus dysgalactiae*	
Enterobacter cloacae		*Vibrio cholerae*	
Enterococcus faecalis		*Vibrio parahaemolyticus*	
Enterococcus faecium		Viridans Streptococci	
Escherichia. coli	O157:H7	*Yersinia enterocolitica*	
Escherichia. coli	O26		
Escherichia. coli	O45	**Viruses:**	
Escherichia. coli	O103	Astrovirus	
Escherichia. coli	O111	Adenovirus	
Escherichia. coli	O121	Coxsackie	
Escherichia. coli	O145	Echovirus	
Escherichia hermannii		Rotavirus	
Helicobacter pylori		Sapovirus	
Lactococcus lactis			
Listeria monocytogenes		**Fungi/Parasites/Other:**	
Morganella morganii		*Bacillus cereus* toxin	
Pleisiomonas shigelloides		*Blastocystis hominis*	
Proteus mirabilis		*Candida albicans*	
Proteus vulgaris		*Cryptosporidium parvum*	
Providencia stuartii		*Entamoeba histolytica*	
Pseudomonas aeruginosa		*Giardia lamblia*	
Pseudomonas fluorescens		Shigatoxin STX1	
Pseudomonas putida		Shigatoxin STX2	
Salmonella agona			

(b) Interference

We recommend that you conduct a comprehensive set of interference studies. Potentially interfering substances include, but are not limited to, the following: blood, mucin, common medications used to relieve constipation or diarrhea, antibiotics, and analgesics; examples of potentially interfering substances are presented in Table 2.

You should evaluate each interfering substance at its potentially highest medically-relevant concentration ("the worst case"); if no significant effect is observed, further testing is not necessary. Interference testing should occur with at least three samples with an analyte concentration above the cutoff and three samples below the cutoff.

Table 2. Substances Recommended for Interference Studies

Substance	Comment
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein
Human blood	Hemoglobin and plasma proteins
Barium sulfate	Contrast medium
Loperamide	Active ingredient in Imodium and several anti-diarrheal drugs
Bismuth subsalicylate	Active ingredient in Pepto-Bismol and several anti-diarrheal drugs
Stearic acid/Palmitic acid (1:1) (fatty acids)	
Amoxicillin	Commonly used antibiotic
Metronidazole	Commonly used antibiotic
Acetaminophen	Commonly used analgesic
Ibuprofen	Commonly used analgesic
Aspartame	Commonly used artificial sweetener

(3) Precision

(a) Within-Laboratory Precision/Repeatability

We recommend that you conduct intra-assay, inter-assay and inter-lot precision studies. You should test sources of variability such as operators, days, and assay runs by testing for a minimum of 12 days (not necessarily consecutive) with 3 replicates of each sample per run. The test panel should consist of 4 - 6 samples spanning the entire range of medically relevant analyte concentration. For your inter-lot evaluation, we recommend that you include at least three lots (as appropriate). Your repeatability study report should include the following information: number of days and runs, number of lots, number of operators, and acceptance criteria applied to the studies. You may perform these studies in-house, i.e., within your own company facility.

For qualitative tests without instrumentation needed, e.g., immunochromatographic tests or lateral flow devices, repeatability studies are usually not necessary.

(b) Between-Laboratory Precision/Reproducibility

The protocol for the reproducibility study may vary slightly depending on the assay format although the sample panel should be the same as described for repeatability studies cited above. As a general guide, we recommend the following approach to reproducibility studies:

- Evaluate the reproducibility of your test at 3 testing sites (this may include two external sites and one in-house site, or 3 external sites).
- Use a five day testing protocol, including a minimum of two runs per day (unless the assay design precludes multiple runs per day), and three replicates of each panel member per run. Have at least two operators at each facility perform the test each day. You should provide training only to the same extent that you intend to train users after marketing the test.

For your testing, we recommend including at least 2 – 3 norovirus subtypes (including GII.4) at a minimum of three levels of viral load that include analyte or output concentrations close to the assay cut-off, e.g.:

- A "high negative" sample (C_5 concentration): a sample with an analyte concentration below the clinical cut-off such that results of repeated tests of this sample are negative approximately 95% of the time (i.e., results are positive approximately 5% of the time).
- A "low positive" sample (C_{95} concentration): a sample with a concentration of analyte just above the clinical cut-off such that results of repeated tests of this sample are positive approximately 95% of the time.
- A "moderate positive" sample: a sample ideally reflecting a clinically relevant concentration. At this concentration one can anticipate positive results approximately 100% of the time, e.g., approximately two to three times the concentration of the clinical cut-off.

When the limit of blank (LoB) is used as a cut-off, then the concentration C_{95} is the same as the limit of detection (LoD) and the zero concentration (no analyte present in sample) is C_5 if LoB is established with Type I error of 5%.[2]

See CLSI document EP15-A2 for additional information on reproducibility study design.[3]

[2] Type I error is the probability of having truly negative samples (i.e., those with zero analyte concentration) give values that indicate the presence of analyte. Type I error is usually set at 5% or less.

[3] Clinical and Laboratory Standards Institute. 2008. User Verification of Performance for Precision and Trueness; Approved Guideline-Second Edition. EP15-A2. [ISBN 1-56238-574-7] Clinical and Laboratory Standards Institute, Wayne PA.

c. Controls

We recommend that you run appropriate controls every day of testing for the duration of the analytical and clinical studies. You may contact FDA for further information regarding appropriate controls. In general, for devices based on immunoassay technology, we recommend that you include the following types of controls:

(1) Negative Controls

A blank sample may serve as a negative control; the blank sample should contain buffer or sample extraction buffer and all of the assay components. This control is used to rule out contamination or increased background in the conjugation reaction. It may not be needed for assays performed in single test disposable cartridges or tubes.

(2) Positive Controls

The positive control contains target capsid proteins, and is used to control the entire assay process including protein extraction (if appropriate), assay performance, and detection. It is designed to mimic a patient specimen and is run as a separate assay concurrently with patient specimens at a frequency determined by a laboratory's Quality System (QS). It may not be needed for assays performed in single test disposable cartridges or tubes. Examples of acceptable positive assay control materials include:

- Specimen from a norovirus infected individual.
- Packaged capsid proteins.

(3) Internal Control

The internal control is a non-target antibody against conjugate antibodies or linker. It controls for integrity of the reagents, carrier matrix, and the presence of inhibitors in the samples. Examples of acceptable internal control materials include anti-mouse IgG antibodies, biotin, streptavidin, and anti-peroxidase. It may only be needed for assays performed in single test disposable cartridges or tubes.

d. Specimen Collection and Handling

The assay should be restricted to fecal specimens collected at a time in the course of disease when norovirus is likely to be isolated from the specimen.

The quality and quantity of the target can be highly dependent on factors such as collection method, handling (e.g., transport, storage times, and temperatures). The testing results that you will provide in your 510(k) should validate that the device maintains acceptable performance (e.g., accuracy, reproducibility) under all the conditions recommended in your labeling. For example, you should assess the effect of recommended storage times and temperatures (including freeze-thaw cycles) on sample stability using an analysis of specimen

aliquots stored and/or transported under your recommended conditions of time and temperature. You should state your acceptance criteria for all specimen stability parameters.

Specimens for pathogen identification should be collected and handled using all applicable state and federal biosafety guidelines. For standard precautions for handling of specimens, refer to the most current editions of the related Clinical and Laboratory Standards Institute (CLSI) documents.[4]

e. Assay Cut-off

Your 510(k) submission should explain how assay cut-off(s) were determined (also see Section 8.g - Test Results). Selection of the appropriate cut-off can be justified by the relevant levels of sensitivity and specificity based on Receiver Operating Curve (ROC) analysis of pilot studies with clinical samples; details regarding ROC analysis are included in CLSI document GP10-A.[5] The performance of your device using the pre-determined cut-off (and equivocal zone, if applicable) should be validated in an independent population consistent with the defined intended use of your device (also see Section 7.f - Clinical Studies).

f. Clinical Studies

You should conduct clinical studies to determine the performance of your device for the specific intended uses of your assay. The approach to specimen collection may differ depending on whether the intended use is as an aid in the diagnosis of norovirus infection in the setting of an individual patient with symptoms of acute gastroenteritis when the individual patient is epidemiologically linked to other patients with symptoms of acute gastroenteritis and/or aid in the identification of norovirus as the etiology of an outbreak of acute gastroenteritis in the setting of epidemiologically linked patients with symptoms of acute gastroenteritis. For the diagnosis of individual patients, specimens should be prospectively collected and tested from individuals representing the intended use population, i.e., patients with signs and symptoms consistent with acute gastroenteritis. Fresh samples are preferred for these studies although it may be possible to supplement fresh samples with prospectively collected archived specimens.[6]

To use prospectively collected archived specimens to evaluate norovirus assays you should demonstrate that sample freezing or other preservation techniques do not affect analyte

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[4] Clinical and Laboratory Standards Institute. 1997. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue. CLSI document M29-A. Clinical and Laboratory Standards Institute, Wayne PA

[5] Clinical and Laboratory Standards Institute. 1997. Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots; Approved Guideline CLSI document GP10-A. Clinical and Laboratory Standards Institute; Wayne, PA.

[6] In this guidance, we define prospectively collected archived specimens as specimens collected sequentially from all patients meeting study inclusion criteria; specimens should not be selectively included based on known results, and all testing should be conducted with investigators completely blinded to any previous results or patient characteristics. Specimens should be as fresh as possible, and, if not fresh (as would be expected for prospectively archived samples), appropriately stored with the storage conditions used described in the submission. The sponsor should show that there is no change in device performance due to banking/freezing/storage of the specimens.

stability, that appropriate archives are selected, and that appropriate measures are taken to identify and remove or mitigate any biases in the study set. If you evaluate the assay using specimens that were archived, you should ensure that the specimens are not selectively utilized, i.e., that all specimens are tested. Samples should be masked during testing to avoid possible bias. If both fresh and archived/frozen samples are tested, we recommend that you analyze the data of these two groups separately. It is likely that samples will be prospectively collected archived specimens for clinical studies of suspected outbreaks.

The protocol for each clinical study performed should be included in the 510(k) submission. Sponsors are strongly encouraged to discuss study protocols with FDA prior to initiation of clinical studies.

The following issues should be also addressed during the design of your clinical trials:

(1) Reference Assays

We recommend that you assess and compare the performance of your device to an appropriate reference standard; due to the numerous norovirus genotypes, a predetermined algorithm based on a composite reference method (i.e., where the results of more than one assay are included as part of the reference standard) is likely to be most appropriate. The composite reference method should include a well-characterized and validated real-time RT-PCR assay with a concomitant conventional RT-PCR assay followed by bi-directional sequencing analysis. Sequencing should be performed on both strands of the amplicon (i.e., bi-directional sequencing), should demonstrate that the generated sequence is at least 200 base pairs of an acceptable quality (e.g., a quality score of 40 or higher as measured by PHRED or similar software packages), and should demonstrate that it matches the reference or consensus sequence.[7,8]

For determining 'Clinical Diagnostic Truth' by the reference method, detection of norovirus by conventional PCR testing with sequencing would be considered evidence of the presence of norovirus regardless of the results of real-time RT PCR testing; specimens positive by real-time RT PCR testing but negative by the initial conventional PCR testing should be tested by conventional PCR testing with different primers from the original testing (e.g., Region B amplification if the original amplification was norovirus Region D). This is illustrated in Table 3 below.

[7] Clinical and Laboratory Standards Institute. 2004. Nucleic Acid Sequencing Methods in diagnostic Laboratory Medicine; Approved Guideline. CLSI document MM9-A [ISBN 1-56238-558-5] Clinical and Laboratory Standards Institute, Wayne PA.

[8] Clinical and Laboratory Standards Institute. 2006. Molecular Diagnostic Methods for Infectious Disease; Approved Guideline. CLSI document MM3-A2 [ISBN 1-56238-596-8] Clinical and Laboratory Standards Institute, Wayne PA.

Table 3. Reference Standard Testing by Composite Reference Method ('Clinical Diagnostic Truth')

Real-Time RT-PCR	Conventional RT-PCR (region D + sequencing)	Conventional RT-PCR (region B + sequencing)*	'Clinical Diagnostic Truth'
Positive	Positive	N/A	Positive
Negative	Positive	N/A	Positive
Positive	Negative*	Positive	Positive
		Negative	Negative
Negative	Negative	N/A	Negative

* In the scenario of a positive real-time RT-PCR assay but negative conventional RT-PCR Region D testing, specimens should have additional testing by conventional RT-PCR Region B testing to establish true positive specimens from false positive real-time PCR results.

If an FDA cleared or approved NAAT-based reference device is not available for norovirus detection, the analytical performance of the NAAT reference assays to be used during clinical trials should be established by analytical sensitivity (LoD) and strain reactivity studies as follows:

(a) Limit of Detection (LoD)

At least one well characterized Genogroup I and one well characterized Genogroup II sample should be tested with the real-time RT-PCR method and the conventional RT-PCR methods with bi-directional sequencing. Testing should consist of analysis of 20% w/v preparations and a further 6 log dilutions, all to be carried out in triplicate for each sample. From the real-time RT-PCR testing, RNA copy numbers should be established for each dilution.

EM testing of your samples should be performed at the lowest dilution (i.e., the initial dilution) to establish the absolute particle number when determining the LoD. This does not need to be repeated for each dilution, but can be extrapolated from the lowest dilution for determining the LoD in particles/gram of fecal tissue.

The LoD for each reference assay should be established based on the number of viral particles and RNA copy number that gives a minimum of 2 out of 3 tests positive in each reference assay. Results should be presented as a table directly comparing results for real-time RT-PCR testing, conventional PCR tests, and EM testing.

(b) Strain Reactivity

Reference assays (both real-time RT-PCR and conventional PCR) should be tested against a panel of well characterized stool samples to demonstrate strain reactivity. The panel should be characterized by bi-directional sequencing of a respective

partial region of the norovirus genome suitable for the identification of both the genogroup as well as the genotype.

It is recommended that the norovirus strains to be tested in the study include:

Genogroup I: GI.1, GI.2, GI.3, GI.4, GI.5, GI.6, GI.7, GI.8
Genogroup II: GII.1-10, GII.12, GII.13, GII.14, GII.15, GII.16, GI1.17
Genogroup IV: GIV.1

All in silico work (e.g., protocols, primer sequences, annealing temperature, etc.) associated with evaluating primer and probe sequences used in the NAAT assays should be submitted to the FDA for review.

The NAAT assays to be used as part of a composite reference method algorithm in the clinical studies (e.g., as shown in Table 3 above) should be in their final formats at the time of LoD and strain reactivity studies, i.e., no modifications or "tweaking" of the assays should be performed after characterization. All protocols and SOPs for the real-time RT-PCR assay and the conventional RT-PCR followed by bi-directional sequencing assays should be provided to the Agency. FDA considers the nucleic acid extraction method (manual or automated), as well as reagents, assay conditions, and instrumentations, as important parts of the NAAT assay. Therefore the final formats of the NAAT assays should include defined nucleic acid extraction methods, assay reagents, assay conditions and instrumentations, etc. Appropriate controls should be incorporated into each NAAT reference assay to be used during clinical studies.

When a NAAT assay that is not cleared or approved by FDA is used as part of the reference algorithm, you should submit to FDA the validation data and literature references used to justify your reference assays in order to demonstrate that the reference assays target conserved regions of the norovirus genome and react broadly in detecting norovirus strains.

It is recommended that you contact the FDA for further information regarding the use of NAAT reference assays and establishing a predetermined algorithm that uses composite reference methods.

(2) Study Protocol

Clinical study protocols should be complete prior to study initiation. At a minimum, protocols should include complete patient inclusion and exclusion criteria, the type and number of specimens needed, study procedures, and a detailed statistical analysis plan. Copies of the original study protocols, protocol modifications, and any other relevant study information should be included in your 510(k) submission.

We encourage sponsors to contact FDA to request a review of their proposed studies and the selection of specimen type as part of the pre-IDE review process. This is particularly

recommended in a situation where different intended uses of the test may be studied or sponsors are planning to submit a 510(k) submission for the first time.

(3) Specimen Type(s)

Fecal specimens are the appropriate sample matrix for studying norovirus. The number of specimens necessary will depend on whether the intended use is for individual patient diagnosis or the investigation of outbreaks; for the latter intended use, the 'unit of analysis' in clinical studies will be each individual outbreak, with a varying number of specimens comprising each outbreak. For studies of individual patient diagnosis, specimens should be collected sequentially from all patients at each study site who meet the specific study inclusion criteria. The total number of samples you should include in your study will depend on anticipated assay performance and the anticipated strain diversity.

We recommend that for the intended use of an aid in the investigation of suspected outbreaks that your 510(k) submission also include a sufficient number of prospectively collected fresh samples to demonstrate the absence of gross differences in performance from archived and fresh specimens.[9] Additional information regarding norovirus outbreak definitions and investigation is available through the Centers for Disease Control and Prevention.[10]

(4) Study Sites

For the intended use of individual patient diagnosis, we recommend that you conduct your studies at a minimum of three different geographical sites representing environments where the device will ultimately be used (e.g., clinical laboratories) and by laboratory personnel likely to perform the test in clinical practice. At least two of the study sites should be locations in the United States. It is recommended that sponsors discuss appropriate study sites for the intended use of outbreak investigation with FDA prior to initiating studies since these studies are more likely to use prospectively archived specimens.

(5) Study Population

Patients enrolled in clinical studies should be patients who meet the study inclusion and exclusion criteria for suspected norovirus infection. For both sporadic cases and potential outbreaks, a minimum set of demographic characteristics including age, sex, date of specimen collection, date of symptom onset, the presence/absence of cardinal symptoms (e.g., nausea, emesis, and/or diarrhea), and whether there has been contact or association with similarly affected individuals should be collected at the time of enrollment.

[9] A separate protocol to may be necessary to study a minimum number of fresh specimens in the setting where the only intended use is for outbreak investigations, and samples from the outbreaks studies are all from prospectively archived sources.

[10] Centers for Disease Control and Prevention. Updated Norovirus Outbreak Management and Disease Prevention Guidelines. MMWR 2011; 60(No. RR-3).

For outbreak investigations, additional information regarding the strategy for sample collection and the origin of the suspected outbreak should be included.

Specimens from patients representing all age groups should be obtained in your clinical studies. (If necessary, including clinical sites that focus on certain patient populations (e.g., pediatric care clinics) can address this.) It is recommended that most samples be collected within 3 days of symptom onset (to insure a sufficient number of positive specimens) although later times of collection may be valuable for estimating device performance over time from symptom onset.

(6) Presentation of Clinical Study Results

Analysis should be based on the intended use, i.e., the unit of analysis should be either by individual specimen or by individual outbreak; for the latter, the definition of the minimum number of reference-method positive patients relative to the number of samples studied for each suspected outbreak should be defined *a priori* in the study protocol.

Study analysis should account for all samples collected. Comparisons of device performance against the reference standard should be included as 2 x 2 tables. Additional analyses should be included for device performance relative to patient characteristics, e.g., subject age, time of specimen collection relative to illness onset, study site, etc. In studies that combine fresh specimens and archived specimens, analyses should compare performance on each specimen type separately and when combined.

All study data should be included in the 510(k) submission as Microsoft Excel, delimited text, or as SAS transport files. Data files should include appropriate annotations or separate codebooks and should include all primary and derived variables, e.g., the result of the clinical reference algorithm for determining the presence of norovirus.[11] Description of the statistical methods applied to the data set should be sufficiently detailed to allow FDA to reproduce from the data files the results reported in the submission.

For the intended use of individual patient diagnosis (i.e., sporadic cases of norovirus infection), in general, study results with a positive agreement of 85% (lower bound of the two-sided 95% confidence interval of 80%) and negative agreement with a lower bound of the two-sided 95% CI greater than 90% for samples collected within the first three days of symptom onset are recommended. For an intended use for the diagnosis of outbreaks, lower estimates of positive agreement may be acceptable and should be discussed with FDA prior to initiating clinical studies.[12]

[11] Separate data files for the analytical studies should also be included in the 510(k) submission.

[12] Several definitions of norovirus outbreaks exist in the epidemiological literature; it is recommended that studies for the intended use of outbreak investigation be discussed with FDA so that this can be specified *a priori* in clinical protocols.

8. Labeling

Human norovirus immunoassay systems, like other devices, are subject to statutory requirements for labeling (including sections 201(n) and 502(a) of the FD&C Act; 21 USC §§ 321(n) and 352(a)). These IVD devices must provide adequate directions for use and adequate warnings and precautions (section 502(f) of the FD&C Act; 21 USC § 352(f)). Specific labeling requirements for all IVD devices are set forth in 21 CFR 809.10; also see 21 CFR 801.119 in which it is stated that IVDs labeled in accordance with 21 CFR 809.10 are deemed to satisfy section 502(f)(1) and 21 CFR part 801. The information described below should assist you in meeting these requirements. These labeling recommendations also help to mitigate the risks identified previously in this guidance to ensure safe and effective use of these devices.

a. Intended Use

The intended use should specify the specific genogroups and genotypes detected by your assay and the qualification that the device is intended as an aid in the diagnosis of norovirus infection in the setting of investigating the cause of acute gastroenteritis outbreaks. Additional qualifications may be appropriate based on the results of clinical trials.

b. Device Description

In the device description, you should briefly describe the test methodology used in this type of device.

c. Procedure

This section should include a general description of the entire analysis procedure, from the collections of patient samples to result reporting.

d. Directions for Use

You should provide clear and concise instructions that delineate the procedures for using the device, and types of controls that will minimize risks of inaccurate results. Instructions should encourage use of additional control measures and testing of control materials to ensure use in a safe and effective manner.

e. Warnings, Precautions, and Limitations

In addition to warnings, precautions, and limitations relevant to your specific device or assay, we recommend including statements such as the following under Limitations, as applicable:

- That the device cannot differentiate between different noroviral genogroups or genotypes, and that only the presence or absence of norovirus is detected (if applicable).

- The listing of the specific genogroups and genotypes that can be detected by the device, and the statement that negative results do not exclude the possibility of infection by a strain not detected by the device.
- That the detection of norovirus does not exclude the presence of other enteric pathogens and that additional testing should be conducted as indicated.
- That negative test results do not exclude the presence of norovirus, and that specimens taken later after the onset of symptoms are more likely to be falsely negative.
- That the detection of norovirus is dependent upon proper specimen collection, handling, transportation, storage and preparation, and that failure to observe proper procedures during any of these steps can lead to incorrect results, most likely to be a falsely negative result. (This may be included at a different section of labeling, e.g., quality control, as appropriate.)
- That device performance has not been established in immunocompromised patients (if appropriate).
- When used for the diagnosis of individual patients the interpretation of positive and negative predictive values is dependent on local disease prevalence at the time of testing.
- That monoclonal antibodies may fail to detect strains of norovirus which have undergone amino acid changes in the target epitope region, and that antigenic changes over time in circulating norovirus strains or the emergence of new norovirus strains may affect test performance.
- That for devices indicated only for outbreak investigations, that the device should not be used for the diagnosis of individual patients.

Certain additional warnings or limitations may be appropriate based on the intended use; for example, for devices intended only for outbreak investigations, there should be a specific limitation/warning that the device should not be used for diagnosis of individual patients.

If positive or negative interference has been reported for any commonly used collection materials or substances that may be endogenously or exogenously introduced into a specimen prior to testing, users should be advised under limitations of the possibility of false negative or false positive results due to such interference.

f. Specimen Collection

We recommend that you state that inadequate or inappropriate specimen collection, storage, number of freeze/thaw cycles and transport are likely to yield false negative test results. It should also be noted in labeling that samples should be collected as soon as possible after symptom onset.

g. Interpretation of Test Results

The interpretation of test results section in the package insert should list all possible assay outputs and determinations of the presence or absence of norovirus. If internal controls are part of the determination of valid positive and negative results, you should provide the interpretation

of each possible control result and a recommendation for how to follow up invalid or indeterminate results.

If your assay has an equivocal zone, you should provide the interpretation and the recommendation for how to follow up with the equivocal result, e.g., whether the equivocal result should be reported as such, or whether testing should be repeated. If repeat testing of an invalid or equivocal result is recommended, you should describe how repeat testing should be performed (e.g., on the same or a different specimen from the same patient).

Final assay results should be reported as positive, negative, or equivocal (as appropriate). Depending on test performance or other device-specific factors, additional qualifications may be appropriate.

9. Postmarket measures

We recommend that you regularly obtain and analyze postmarket data to ensure the continued reliability of your device for detecting different norovirus strains as noroviruses may evolve over time.[10] This is particularly true if new noroviral strains emerge, or if noroviruses that are less common at the time of device clearance become more prevalent. Postmarketing data should address the clinical performance of your device under the new conditions.

To demonstrate how you will address this aspect of the special control, we recommend that you provide a plan with your 510(k) submission that describes how you intend to assure that the performance characteristics of your test remain unchanged over time. This plan is likely to include periodic testing of highly prevalent noroviral stains at defined time intervals with your device. FDA will evaluate whether this plan will help to mitigate the risks presented by the device and therefore help to provide continued reasonable assurance of the safety and effectiveness of the device.