

# **Guidance for Industry and FDA Staff**

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## **Class II Special Controls Guidance Document: RNA Preanalytical Systems (RNA Collection, Stabilization and Purification Systems for RT-PCR used in Molecular Diagnostic Testing)**

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**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Devices and Radiological Health**

**Office of In Vitro Diagnostic Device Evaluation and Safety  
Division of Immunology and Hematology Devices**

# Preface

## Public Comment

Written comments and suggestions may be submitted at any time for Agency consideration to Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. Alternatively, electronic comments may be submitted to <http://www.fda.gov/dockets/ecomments>. When submitting comments, please refer to Docket No [2005D-0264] Comments may not be acted upon by the Agency until the document is next revised or updated.

## Additional Copies

Additional copies are available from the Internet at: <http://www.fda.gov/cdrh/oivd/guidance/1563.pdf>, or to receive this document by your fax machine, call the CDRH Facts-On-Demand system at 800-899-0381 or 301-827-0111 from a touch-tone telephone. Press 1 to enter the system. At the second voice prompt, press 1 to order a document. Enter the document number (1563) followed by the pound sign (#). Follow the remaining voice prompts to complete your request. For questions regarding the use or interpretation of this guidance contact: Uwe Scherf at 240-276-0496 or by email at [uwe.scherf@fda.hhs.gov](mailto:uwe.scherf@fda.hhs.gov).

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## **Class II Special Controls Guidance Document: RNA Preanalytical Systems (RNA Collection, Stabilization and Purification Systems for RT-PCR used in Molecular Diagnostic Testing)**

*This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.*

### **1. Introduction**

This guidance document was developed as a special controls guidance to support the classification of RNA preanalytical systems (RNA collection, stabilization and purification systems) into class II (special controls). RNA preanalytical systems are devices intended to collect, store, and transport patient specimens, and stabilize intracellular RNA from the specimens, for subsequent isolation and purification of the intracellular RNA for RT-PCR (real-time polymerase chain reaction) used in *in vitro* molecular diagnostic testing. The device may consist of sample collection devices, nucleic acid isolation and purification reagents, and processing reagents/equipment (tubes, columns, etc.). It also may contain instruments for automation of the nucleic acid isolation and purification steps.

This guidance is issued in conjunction with a Federal Register notice announcing the classification of RNA preanalytical systems. Any firm submitting a premarket notification (510(k)) for a RNA preanalytical system will need to address the issues covered in this special controls guidance document. However, the firm need only show that its device meets the recommendations of the guidance or in some other way provides equivalent assurances of safety and effectiveness.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidance documents describe the Agency's current thinking on a topic and should be viewed

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only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance documents means that something is suggested or recommended, but not required.

## **The Least Burdensome Approach**

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be marketed. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to follow the statutory and regulatory criteria in the manner suggested by the guidance and in your attempt to address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe that there is a less burdensome way to address the issues, you should follow the procedures outlined in the document, “A Suggested Approach to Resolving Least Burdensome Issues.” It is available on our Center web page at: <http://www.fda.gov/cdrh/modact/leastburdensome.html>.

## **2. Background**

FDA believes that special controls, when combined with the general controls, will be sufficient to provide reasonable assurance of the safety and effectiveness of a RNA preanalytical system. A manufacturer who intends to market a device of this generic type should (1) conform to the general controls of the Federal Food, Drug, and Cosmetic Act (the Act), including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific risks to health associated with the device identified in this guidance, and (3) obtain a substantial equivalence determination from FDA before marketing the device.

This guidance document identifies the classification regulation and product code for RNA collection, stabilization, and purification systems. (Refer to Section 4 – **Scope**). In addition, other sections of this guidance document identify 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 RNA preanalytical systems 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 other FDA documents on this topic, such as the **510(k) Manual - Premarket Notification: 510(k) - Regulatory Requirements for Medical Devices**, <http://www.fda.gov/cdrh/manual/510kprt1.html>.

As explained in “**The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**”<sup>1</sup>, a manufacturer may submit either a Traditional 510(k) or an Abbreviated 510(k). FDA believes an Abbreviated 510(k) provides the least burdensome means of demonstrating substantial equivalence for a new device, particularly when FDA has issued a guidance document that provides recommendations on what should be addressed in a submission for the device. Alternatively, manufacturers considering modifications to their own cleared devices may lessen the regulatory burden by submitting a Special 510(k).

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<sup>1</sup> <http://www.fda.gov/cdrh/ode/parad510.html>

### **3. The Content and Format of an Abbreviated 510(k) Submission**

An Abbreviated 510(k) submission must include the required elements identified in 21 CFR 807.87, including the proposed labeling for the device sufficient to describe the device, its intended use, and the directions for its use. In an Abbreviated 510(k), FDA may consider the contents of a summary report to be appropriate supporting data within the meaning of 21 CFR 807.87(f) or (g); therefore, we recommend that you include a summary report. The report should describe how this guidance document was used during the device development and testing and the methods or tests used. The report should also include a summary of the test data or description of the acceptance criteria applied to address the risks identified in this document, as well as any additional risks specific to your device. This section suggests information to fulfill some of the requirements of 21 CFR 807.87 as well as some other items that we recommend you include in an Abbreviated 510(k).

#### **Coversheet**

The coversheet should prominently identify the submission as an Abbreviated 510(k) and cite the title of this guidance document.

#### **Proposed labeling**

Proposed labeling should be sufficient to describe the device, its intended use, and the directions for its use. (Refer to Section 8 for specific information that you should include in the labeling for this type of device.)

#### **Summary report**

We recommend that the summary report contain the following:

- A description of the device and its intended use. We recommend that the description include a complete discussion of the performance specifications and, when appropriate, detailed, labeled drawings of the device. You should also submit an "indications for use" enclosure.<sup>2</sup>
- A description of the device design. We recommend that the description include a complete discussion of the performance specifications and, when appropriate, detailed, labeled drawings of the device.
- Identification of the Risk Analysis method(s) used to assess the risk profile in general, as well as the specific device's design and the results of this analysis. (Refer to Section 5 for the risks to health generally associated with the use of this device.)

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<sup>2</sup> Refer to <http://www.fda.gov/cdrh/ode/indicate.html> for the recommended format.

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- A discussion of the device characteristics that address the risks identified in this class II guidance document, as well as any additional risks identified in your risk analysis.
- A brief description of the test method(s) you have used or intend to use to address each performance aspect identified in Sections 6 and 7 of this guidance document. If you follow a suggested test method, you may cite the method rather than describing it. If you modify a suggested test method, you may cite the method, but should provide sufficient information to explain the nature of and reason for the modification. For each test, you may either (1) briefly present the data resulting from the test in clear and concise form, such as a table, **or** (2) describe the acceptance criteria that you will apply to your test results.<sup>3</sup> (See also 21 CFR 820.30, Subpart C - Design Controls for the Quality System Regulation.)
- If you choose to rely on a recognized standard for any part of the device design or testing, you may include either: (1) a statement that testing will be conducted and meet specified acceptance criteria before the product is marketed, or (2) a declaration of conformity to the standard.<sup>4</sup> Because a declaration of conformity is based on results from testing, we believe you cannot properly submit a declaration of conformity until you have completed the testing the standard describes. For more information, please refer to section 514(c)(1)(B) of the Act and the FDA guidance, **Use of Standards in Substantial Equivalence Determinations; Final Guidance for Industry and FDA**, <http://www.fda.gov/cdrh/ode/guidance/1131.html>.

If it is not clear how you have addressed the risks identified by FDA or additional risks identified through your risk analysis, we may request additional information about aspects of the device's performance characteristics. We may also request additional information if we need it to assess the adequacy of your acceptance criteria. (Under 21 CFR 807.87(l), we may request any additional information that is necessary to reach a determination regarding substantial equivalence.)

As an alternative to submitting an Abbreviated 510(k), you can submit a Traditional 510(k) that provides all of the information and data required under 21 CFR 807.87 and described in this guidance. A Traditional 510(k) should include all of your methods, data, acceptance criteria, and conclusions. Manufacturers considering modifications to their own cleared devices should consider submitting Special 510(k)s.

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<sup>3</sup> If FDA makes a substantial equivalence determination based on acceptance criteria, the subject device should be tested and shown to meet these acceptance criteria before being introduced into interstate commerce.

<sup>4</sup> See Required Elements for a Declaration of Conformity to a Recognized Standard (Screening Checklist for All Premarket Notification [510(K)] Submissions), <http://www.fda.gov/cdrh/ode/reqrecstand.html>.

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## 4. Scope

The scope of this document is limited to the following device as described in 21 CFR 866.4070 (product code NTW):

21 CFR 866.4070 RNA Preanalytical Systems.

RNA Preanalytical Systems are devices intended to collect, store, and transport patient specimens, and stabilize intracellular RNA from the specimens, for subsequent isolation and purification of the intracellular RNA for RT-PCR used in *in vitro* molecular diagnostic testing.

These devices may consist of sample collection devices, nucleic acid isolation and purification reagents, and processing reagents/equipment. The system may also contain instruments for automation of the nucleic acid isolation and purification steps.

## 5. Risks to Health

Failure of the system during specimen collection, or during RNA stabilization or purification could yield an RNA sample of low quality and quantity. Low quality RNA, when tested, could result in falsely low or falsely high RNA transcript signal levels leading to inaccurate diagnosis and/or improper patient management. Low quantity of RNA could render the samples unusable for downstream RT-PCR applications; specimens would need to be recollected, causing possible delay in diagnosis. In addition, depending on specimen type, recollection could pose additional patient risk (e.g., tissue biopsy). The degree of risk varies depending on the disease or condition/stage being diagnosed or managed. Results of RNA testing should always be considered in conjunction with other clinical factors.

In the table below, FDA has identified the risks to health generally associated with the use of a RNA preanalytical system addressed in this document. The measures recommended to mitigate the identified risks are described in this guidance document, as shown in the table below. You should 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 the risks identified in this 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 risk	Recommended mitigation measures
Inaccurate Results and Improper Patient Management	Sections 6, 7
Delay in Diagnosis	
Need for patient specimen recollection	



## **6. Device Description**

In your 510(k), you should identify the regulation, the product code, and a legally marketed predicate device. In order to help FDA efficiently review all the aspects of your device compared with the predicate, you should include a table that outlines the similarities and differences between the predicate and your device.

Key issues in the review of a new device are the specific intended use, the type of specimens tested, and the technology utilized. You may submit appropriate peer-reviewed literature references relevant to the technology of the device in addition to the descriptive information to adequately describe the new instrumentation. You should include the following descriptive information to adequately characterize RNA preanalytical systems.

### **Intended Use**

You should clearly describe the intended use of the device. The intended use should specify the components of the device (e.g. tubes, purification reagents), the type of specimen the device is used for, the intended application (e.g., collection, storage, transport, stabilization) and the analyte processed (e.g., intracellular or viral RNA). You should also list the type of molecular diagnostic test (RT-PCR, bDNA, etc.) used in conjunction with the device.

### **Description of Methodology**

We recommend that you provide a thorough explanation of all aspects of the system methodology. This could include, but is not limited to, the following:

- How the system is designed to carry out its functions related to collection, stabilization, or purification.
- The specimen type(s) that may be used with the system.
- Any instrument components provided, or recommended, and their function in the system.

## **7. Performance Characteristics**

The quality of extracted RNA is essential for high-quality RT-PCR diagnostic assays. You should conduct statistically-based studies using clinical samples to demonstrate RNA stability, purity, integrity, yield, repeatability, reproducibility, and suitability for use in RT-PCR assays according to claims stated in the product labeling.

### **Specific Study Recommendations**

#### **Collection Parameters :**

##### *Collection volume for blood specimens*

You should substantiate the draw volume you claim in your labeling by assessing whether the blood collection device can maintain between 90% and 110% of the nominal collection volume, over the

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product shelf life stated in your labeling. Examples of the study design and parameters for collection volume are described in detail in “Tubes and Additives for Venous Blood Specimen Collection; Approved Standard-Fifth Edition” (2003), Clinical and Laboratory Standards Institute (CLSI), Document H1-A5.

### Other specimen sources (cells, tissues)

We recommend that you document and justify your choice of specimen collection and storage methods. For questions regarding specimen collection you should contact the appropriate divisions in the Office of In Vitro Diagnostic Device Evaluation and Safety.

## **RNA quality assessment:**

### RNA yield

You should establish the minimum acceptable RNA yield from the system and provide data to demonstrate that this is consistently attainable. RNA yield is usually defined as the absorbance at 260 nm ( $A_{260}$ ) diluted in Tris Cl, pH 7.5, using a spectrophotometer.

### RNA stability

You should substantiate statements in your labeling about specimen storage and transport by assessing whether the system can maintain acceptable performance (e.g., RNA yield, purity, integrity, and minimal changes in gene transcript profiles) over the specimen storage times and temperatures you recommend. An appropriate study would include an analysis of RNA isolated from blood specimens across the conditions of time, temperature, or number of freeze/thaw cycles that you recommend to users of the system. You should state the criteria for an acceptable range of RNA yield, purity, integrity, and performance in specific RT-PCR diagnostic test methods.

### RNA purity and integrity

You should establish the acceptable range of RNA purity. This is usually defined as  $A_{260}/A_{280}$  at pH 7.5. You should provide data that demonstrates the overall integrity of the isolated RNA. You should also establish the acceptable level of genomic DNA (gDNA) in the purified RNA samples and submit data to support this.

### Suitability for RT-PCR and assay validation

To demonstrate that the system can be used for RT-PCR used in molecular diagnostic testing, you should include testing of the system with RT-PCR assay(s). We recommend using FDA cleared or approved assays, if available. If the assay(s) is not FDA cleared or approved, you should include validation of the assay in your 510(k). You should submit all data to support the analytical performance characteristics of the assay. You should consult the Office of In Vitro Diagnostic Device Evaluation and Safety for advice if you are considering using an uncleared assay for validation of your device.

Suitability studies should include testing to demonstrate that no reagents in the RNA pre-analytical System interfere with nucleic acid amplification.

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### **Device stability:**

We recommend that you include device stability testing to support the shelf life claim (e.g., reagents and other components). [See EN 13640 - 2002 Stability testing of *in vitro* diagnostic reagents.]

### **Precision (Repeatability/Reproducibility):**

You should fully examine the reproducibility of your RNA collection, stabilization and purification system. “Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline” (2004) CLSI Document EP5-A2, “User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline” (2002), CLSI Document EP12-A, and “Protocols for Determination of Limits of Detection of Quantitation: Approved Guideline” (2004), CLSI Document EP17-A include guidelines for experimental design, computations, and a format for stating performance claims. You should include the following in your study design:

- Design the study so that you can characterize intra-, inter- and total assay reproducibility.
- Use appropriate test specimens containing varying RNA concentrations, similar to the concentrations you recommend for your device. We recommend that you include at least 10 specimens. You should test all sample types (e.g. whole blood, buccal swabs, tissues, or other intended use matrices) that can be used with the device.
- Ensure that samples used in reproducibility testing are processed from actual patient samples, at the test site. Simulated samples can be used in cases where patient sample are not available. Processing should mimic the procedure you recommend in the device labeling.
- Include 3 or more sites with multiple operators at each site. Operators should reflect potential users of the assay, in terms of education and experience. If training will be necessary for users to perform the test once it is marketed, you should provide information on operator training. If such training is not expected to be provided for users, you should not provide additional training (other than the package insert) at the testing sites.
- Characterize the reproducibility and repeatability of RNA yield for your system. The study should be carried out by at least three qualified operators. RNA yield from each of the devices should be measured by a spectrophotometric method. You should report mean, standard deviation, and coefficients of variation for each specimen and for each operator (as a measure of repeatability), as well as over all operators and sites (as a measure of reproducibility).
- Establish the reproducibility and repeatability of RNA purity and integrity. At least three operators, employing three sets of laboratory equipment, should carry out this study. RNA yield from each of the devices should be measured by a

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spectrophotometric method. You should demonstrate that the results are within the established range of RNA purity stated in your labeling.

- Establish that reproducible RNA transcript signal levels can be obtained regardless of variation in device operator, lot, day, laboratory, etc.
- Ensure that procedures used in the reproducibility studies are the same as the procedure that you will recommend to users in the package insert.
- Include multiple product lots, and multiple instruments (if instruments are part of the test system), to adequately test the expected performance of the system.

In the study design description in your 510(k), you should identify which factors (e.g., reagent lots, operators) were held constant and which were varied during the evaluation, and describe the computational methods and statistical analyses used to evaluate the data.

#### **Instrumentation and software (if applicable)**

If the system includes instrument(s) that are used to automate the RNA isolation and purification steps, the submission should include all required information as detailed in the FDA/CDRH “Guidance for FDA Reviewers and Industry: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices” document available at <http://www.fda.gov/cdrh/ode/guidance/337.pdf>. You should include a copy of the instrument manual for specified instrumentation. All of the analytical performance data, including the repeatability and reproducibility data, should be generated using the automated system.

## **8. Labeling**

The premarket notification should include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). The following suggestions are aimed at assisting you in preparing proposed labeling that satisfies the requirements of 21 CFR 807.87(e). Although final labeling is not required for 510(k) clearance, final labeling must comply with the requirements of 21 CFR 809.10 before an in vitro diagnostic device is introduced into interstate commerce.

#### **Directions for use**

You should provide clear and concise instructions on the use of the system from specimen collection (e.g. blood) to RNA isolation and purification. You should include work-flow recommendations for the RNA isolation and purification steps. You should include pictograms and flow diagrams, wherever appropriate.

#### **Limitations**

We recommend that you address the limitations of your system with statements in the labeling, such as the following:

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- Performance characteristics have not been established for all transcripts. The user is responsible for establishing appropriate system performance characteristics for other target transcripts.
- The system is intended for the purification of intracellular RNA from human whole blood. It is not for the purification of genomic DNA or viral nucleic acids.
- The system is intended for purification of intracellular RNA from human whole blood with leukocytes counts between  $4.8 \times 10^6 - 1.1 \times 10^7$  leukocytes/ml.

#### **Stability**

We recommend that you include product storage conditions and sample stability information in the labeling.

#### **Performance Characteristics**

We recommend that you provide product performance information such as RNA purity, yield, repeatability, reproducibility, and stability of gene transcript levels in the labeling. We recommend that you describe all relevant aspects of the protocols used to establish your performance characteristics, including materials used and results. We also recommend that you provide graphic representations of the results.

#### **User Manual**

If software is a component of your device, we recommend that you provide a user manual that addresses all components of the instrumentation for RNA pre-analytical systems. Your user manual should provide an adequate description of the role of the software, the user interface with the software, as well as results of performance testing to demonstrate that the software functions as designed. We recommend pictorial representations of computer screens, graphical user interfaces (GUIs), and other elements that aid the user in correctly using the software.

The user manual, where possible, should also include descriptions of how the user can recognize incorrect operation or failure of the instrumentation, and a troubleshooting guide.