

# **Overview of Labeling Claims for Donor Screening Tests Using Cadaveric Blood Specimens**

**Perspectives on *In Vitro* Diagnostic Devices Regulated by the  
Office of Blood Research and Review Public Workshop**

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15 July 2019

# Overview

- Background
- Current recommendations for obtaining a labeling claim for donor screening tests using cadaveric blood specimens

# Background

- July 29, 1997, FDA published the Final Rule, “Human Tissue Intended for Transplantation,” 21 CFR part 1270
  - Required donor screening and testing to help prevent the transmission of certain infectious diseases through human tissues used in transplantation.
  - §1270.21(d)
    - Required donor specimens to be tested for HIV-1/2, Hepatitis B, and Hepatitis C, using FDA-licensed donor screening tests in accordance with manufacturers’ instructions
    - FDA licensed screening tests labeled for cadaveric specimens must be used when available.

# Background

- Cadaveric blood specimens from non-heart beating donors are metabolically and biochemically different than blood specimens from living donors
- Cadaveric blood specimens exhibit higher levels of inhibiting substances (*e.g.*, hemolysis, lipemia, *etc.*) compared to living donors
  - Interfering substances can influence assay performance, potentially leading to erroneous test results

1. Federal Register of July 29, 1997 (62 FR 40429)

2. Guidance for Industry: Availability of Licensed Donor Screening Tests for Cadaveric Blood Specimens, June 2000.

# Background

- Due to the changes that occur post-mortem, to obtain a cadaveric labeling claim for a donor screening test, additional studies and validation are needed for cadaveric blood specimens

1. Federal Register of July 29, 1997 (62 FR 40429)
2. Guidance for Industry: Availability of Licensed Donor Screening Tests for Cadaveric Blood Specimens, June 2000.

# Background

- Recognizing the need for appropriately evaluated and specifically labeled test kits, FDA issued letters in 1995 to manufacturers of donor screening tests that:
  - Introduced the subject of expanding the indication for use of blood donor screening tests to include testing of cadaveric blood specimens and
  - Suggested a minimum protocol for testing to follow for validation of assays

1. Guidance to Industry: Recommendations for Obtaining a Labeling Claim for Communicable Disease Donor Screening Tests Using Cadaveric Blood Specimens from Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)
2. Federal Register of July 29, 1997 (62 FR 40429)
3. Guidance for Industry: Availability of Licensed Donor Screening Tests for Cadaveric Blood Specimens, June 2000.

# Background

- Published in June 2000
- Announced availability of two licensed donor screening tests labeled for use with cadaveric (post-mortem) blood specimens
- Pursuant to 21 CFR 1270.21(d), FDA expects testing of cadaveric samples for HIV-1, HIV-2 and Hepatitis B should be performed using test kits specifically labeled for screening of cadaveric blood specimens...not later than January 31, 2001.”

## Guidance for Industry

### Availability of Licensed Donor Screening Tests Labeled for Use with Cadaveric Blood Specimens

Comments and suggestions regarding this document may be submitted at anytime to Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the title of this guidance document.

Additional copies of this guidance document are available from the Office of Communication, Training and Manufacturers Assistance (HFMA-40), 1401 Rockville Pike, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at <http://www.fda.gov/cber/guidelines.htm>

For questions on the content of the draft document contact Dr. Ruth Solomon, 301-827-0078.

U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Biologics Evaluation and Research (CBER)  
June 2000

Note: Between 2001 and 2004, a number of additional assays were approved or licensed with a cadaveric claim



# Background

- In 1997, FDA announced a proposed tiered, risk-based approach for regulating all human cells, tissues, or cellular or tissue-based products (HCT/Ps)
- Broader scope included previously unregulated cells and tissues
- Issued through rulemaking in 3 parts - 21 CFR part 1271:
  - HCT/P Establishment Registration and Listing (Issued January 19, 2001)
  - **Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (Issued on May 25, 2004)**
  - Current Good Tissue Practice for HCT/P Establishments; Inspection and Enforcement (Issued on November 24, 2004)
- Entire 21 CFR part 1271 became effective on May 25, 2005



# Background

## 21 CFR Part 1271

Effective May 25, 2005

21 CFR Part 1271	Includes
Subpart A: General Provisions	Definitions; criteria for regulatory pathway determination (e.g., 361 HCT/P vs. 351 HCT/P)
Subpart B: Establishment Registration and Listing	Requirements for establishment registration and listing products they manufacture
<b>Subpart C: Donor Eligibility</b>	<b>Requirements for donor screening and testing for “relevant communicable disease agents and diseases,” and for making a donor eligibility determination</b>
Subpart D: Current Good Tissue Practice (CGTP)	Handling and process controls to prevent the introduction, transmission, or spread of communicable diseases
Subpart E: Additional Requirements	Reporting adverse reactions and HCT/P deviations; labeling
Subpart F: Inspection and Enforcement	Inspection; import; orders of retention, recall, destruction, and cessation of manufacturing



# Background

- §1271.80(c)-- a donor specimen must be tested:
  - Using appropriate FDA-licensed, approved, or cleared donor screening tests, according to manufacturer's instructions, to adequately and appropriately reduce the risk of transmission of relevant communicable disease agents or diseases (RCDADs).
  - Establishments must also use a test specifically labeled for cadaveric specimens instead of a more generally labeled test “when applicable and when available”.

Note: A “more generally labeled test” includes tests labeled for screening donors of blood and blood products as well as those specifically labeled for screening of other living donors.

- Other living donors include reproductive cell and tissue donors, organ donors, donors of hematopoietic progenitor/stem cells (HPCs), and donor lymphocytes for infusion, and cellular and tissue donors

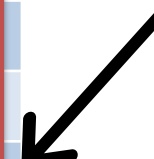
# Background

Donor Testing: §1271.85

Test a donor specimen for evidence of infections due to:

Pathogen	Donors
HIV 1/2	All
HBV	All
HCV	All
<i>T. pallidum</i>	All
WNV	Living**
HTLV I/II	Viable, leukocyte-rich
CMV*	Viable, leukocyte-rich
<i>Chlamydia trachomatis</i>	Reproductive***
<i>Neisseria gonorrhoeae</i>	Reproductive***

Tests not included in 21 CFR part 1270



\* CMV is not an RCDAD. You must establish and maintain a standard operating procedure governing the release of an HCT/P from a donor whose specimen tests reactive for CMV.

\*\* WNV testing: June 1<sup>st</sup> – October 31<sup>st</sup> in U.S. (50 states + D.C.); year-round elsewhere

\*\*\* Unless recovered using a method that ensures freedom from contamination infectious disease organisms that may be present in the genitourinary tract

# Adequate and Appropriate Tests

## HIV-1

- FDA licensed screening test:
  - Anti-HIV-1 or combo test for anti-HIV-1 and anti-HIV-2, **AND**
  - NAT test for HIV-1 or combination NAT test

## HBV

- FDA licensed screening test:
  - Hepatitis B surface antigen (HBsAg),
  - Total antibody to Hepatitis B core antigen (IgG & IgM; anti-HBc), **AND**
  - NAT test for HBV

## *Chlamydia trachomatis*

- FDA cleared diagnostic test for detection of:
  - NAT test for CT in an asymptomatic, low-prevalence population

## *Neisseria gonorrhoeae*

- FDA cleared diagnostic test for detection of:
  - NAT test for NG in an asymptomatic, low-prevalence population

## HIV-2

- FDA licensed screening test:
  - Anti-HIV-2 or combo test for anti-HIV-1 and HIV-2

## HCV

- FDA licensed screening test:
  - Anti-HCV, **AND**
  - NAT test for HCV or combination test

## CMV

- FDA cleared screening test:
  - Anti-CMV, total IgG and IgM

## *Treponema pallidum*

- FDA cleared screening test:
  - Nontreponemal or treponemal

## HTLV-I/II

- FDA licensed screening test:
  - Anti-HTLV-I/II

## WNV

- FDA licensed screening test:
  - NAT test for WNV

DE Guidance, sections VI.A.-B.  
Syphilis Guidance  
WNV Guidance  
HBV NAT Guidance

**Recommendations for Obtaining a Labeling Claim for Communicable Disease Donor Screening Tests Using Cadaveric Blood Specimens for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)**

# Cadaveric Labeling Claim

- Published November 2004
  - Issued in response to blood donor screening test kit manufacturers' requests for information on studies needed to support modifying the indicated use of a labeling claim to include testing cadaveric (non-heart beating) blood specimens from HCT/P donors
- Recommendations for:
  - Sensitivity, specificity and reproducibility studies
    - Sample size, number of test kit lots
  - Plasma dilution issues
  - Including specimen collection times and hemolyzed specimens

## Guidance for Industry

### **Recommendations for Obtaining a Labeling Claim for Communicable Disease Donor Screening Tests Using Cadaveric Blood Specimens from Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)**

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(4)(i). Submit comments on this guidance at any time to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at <http://www.fda.gov/cber/guidelines.htm>.

For questions on the content of this guidance, contact Melissa Greenwald, M.D., at 301-827-2002.

U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Biologics Evaluation and Research  
November 2004

# Cadaveric Labeling Claim: Specificity Studies

- Recommendation when matched pairs of pre- and post-mortem serum or plasma specimens **are available** for testing from each donor:
  - Clinical Specificity:
    - How often test is negative in non-diseased donors
    - Test minimum of 50 **paired** specimens (1 pre-mortem and 1 post-mortem specimen each from the same donor, but test minimum of 50 different donors)
    - Determine if statistically significant difference exists between pre-mortem and post-mortem specimens based on frequency of false positive results

# Cadaveric Labeling Claim: Specificity Studies

- Recommendation when matched pairs of pre- and post-mortem serum or plasma specimens **are available** for testing from each donor:
  - Analytical Specificity
    - Measures a test's ability to exclusively identify a target substance rather than different substances
    - Determine if a statistically significant difference exists between pre- and post-mortem specimens based on signal strength



# Cadaveric Labeling Claim: Sensitivity Studies



- Recommendation when matched pairs of pre- and post-mortem serum or plasma specimens **are available** for testing from each donor:
  - Test at least 50 paired reactive specimens, 1 pre- and post-mortem specimen from **same donor**
  - Clinical Sensitivity:
    - Measures how often test is positive in diseased donors
      - Determine if statistically significant difference exists between pre- and post-mortem specimens based on false negative results
  - Analytical Sensitivity:
    - Ability of test to detect low concentration of a substance
      - Determine if statistically significant difference exists between pre- and post-mortem specimens based on signal strength and end-point dilutions of positive specimens

# Cadaveric Labeling Claim: Specificity Studies

- Recommendation when matched pairs of pre-and post-mortem serum or plasma specimens **are not available** for testing from each donor:
  - Concurrently test at least 50 different cadaveric (non-heart beating or post-mortem) specimens from at least 50 different cadaveric donors and an equal number of random living donor specimens using same test kit lots
  - Clinical Specificity:
    - Determine if statistically significant difference exists between cadaveric and living donors based on frequency of false positive results
  - Analytical Specificity:
    - Determine if statistically significant difference exists between cadaveric and living donor specimens based on signal strength

# Cadaveric Labeling Claim: Sensitivity Studies



- Recommendation when matched pairs of pre- and post-mortem serum or plasma specimens **are not available** for testing from each donor:
  - Analytical Sensitivity:
    - Minimum of 50 non-reactive cadaveric specimens from 50 different cadaveric donors (non-heart beating) and an equal number of random living donor specimens, using same kit lots
    - Both types of specimens spiked with the infectious disease marker spiked at a potency near the assay's cut-off
    - Use a minimum of 5 individual positive sources for the spiking experiment
    - Determine if a statistically significant difference exists between the spiked living donor specimens and spiked cadaveric specimens based on signal strength

# Cadaveric Labeling Claim: Reproducibility Studies

- Minimum 20 cadaveric donor specimens and 20 living donor specimens spiked to be reactive near the assay's cutoff
  - Confirmed true positives may be excluded from study
- Test each specimen individually:
  - In 6 separate runs on 6 separate days
  - Using 3 different test kit lots
  - This will give a total of 18 data points per specimen
- It is recommended that specimens are tested on 6 separate days and stored at 4°C to avoid repeated freezing/thawing

# Cadaveric Labeling Claim: Reproducibility Studies

- Determine if a statistically significant difference exists between the coefficients of variations (CV) of cadaveric specimens versus those of living donors

Note: Sponsors also provide:

- %CV for within-run, between-run, between-day, between-lot, between-site, instrument to instrument, total %CV, *etc.*
- Within-day and within-laboratory %CV if indicated
- Instrument lot to lot reproducibility is required for use with blood donor specimens from living donors and is helpful for use with cadaveric blood specimens

# Cadaveric Labeling Claim: Plasma Dilution



- Prior to including a cadaveric specimen in any study, assess for plasma dilution:
  - Transfusion or infusion of fluid might dilute plasma, making test results unreliable
    - We recommend you test a specimen taken from the donor before the transfusion or infusion, or
    - If an adequate pre-transfusion/infusion specimen is not available, you may use an appropriate algorithm designed to evaluate volumes administered in the 48 hours prior to specimen collection, to determine whether plasma dilution is or is not sufficient to affect test results

# Cadaveric Labeling Claim: Plasma Dilution

- In an adult donor, if blood loss is known or suspected, plasma dilution may have occurred, particularly in setting of:
  - Transfusion/infusion of more than 2000 mL of blood or colloids within 48 hours, or
  - More than 2000 mL of crystalloids within 1 hour, or
  - Any combination thereof, prior to the collection of the blood specimen.



# Cadaveric Labeling Claim: Cadaveric Specimen Collection

- It is recommended that you note the time between death and specimen collection
  - To accurately document test kit performance, the time cadaveric specimens are taken should incorporate the full range of time points typically encountered during tissue recovery (0-24 hours)
- It is recommended that you also include hemolyzed specimens in each cadaveric study
  - A large percentage of cadaveric specimens are hemolyzed due to post-mortem biologic processes; hemolysis can interfere with assay results
  - Quantify the degree of hemolysis, if possible





# Cadaveric Labeling Claim: Specimen Collection



- The guidance document recommends collecting information about storage and handling conditions of both living donor specimens and cadaveric donor specimens

Note: In addition to the studies recommended in the guidance, many sponsors also include validation studies of a range of acceptable storage conditions:

- Stability studies or cadaveric storage studies at different temperatures over different time periods
- These tests inform the storage and handling conditions included in the package insert

# Keys Issues for Consideration:

- What is the appropriate sample size?
  - Guidance document recommends the minimum sample size for each study
  - It is up to the test kit manufacturer to determine the optimal sample size for their studies
- How many test kit lots to include in the studies?
  - We recommend you include at least 3 test kit lots in all cadaveric studies

# Summary

- Obtaining Labeling Claim for Use of Cadaveric Blood Specimens with Donor Screening Tests
  - Recommendations to test kit manufacturers on preparing protocols for cadaveric studies
    - Sensitivity and Specificity studies
    - Reproducibility studies
    - Assessment for Plasma dilution
    - Hemolysis
    - Storage and handling information

# Summary



- Sensitivity and specificity:
  - Minimum 50 cadaveric and 50 living donor specimens
    - Small number due to limited availability and least burdensome principle
  - For analytical sensitivity, minimum of 5 individual positive sources for the spiking experiment, spiked at a potency near assay's cut-off
  - Depending on the requested claim, studies may include:
    - 50 cadaveric serum specimens and/or 50 cadaveric EDTA plasma specimens vs. other anticoagulants
    - These may be labeled as 50 cadaveric blood specimens collected in serum collection tubes and/or 50 cadaveric blood specimens collected in EDTA plasma collection tubes
  - Determine if a statistically significant difference exists between cadaveric and living donor specimens
- Reproducibility
  - Minimum 20 cadaveric and 20 living donor specimens spiked to be reactive near the assay's cutoff, 6 separate runs, 6 separate days
  - Determine a statistically significant difference exists between %CV between cadaveric and living donor blood specimens
- Include 3 test kit lots in all studies

# Helpful Resources

- Tissue & Tissue Products Homepage
  - <https://www.fda.gov/BiologicsBloodVaccines/TissueTissueProducts/default.htm>
- 21 CFR part 1271
  - [http://www.ecfr.gov/cgi-bin/text-idx?SID=ae1deecc79a9f185d48af015ae277f5d&mc=true&tpl=/ecfrbrowse/Title21/21cfr1271\\_main\\_02.tpl](http://www.ecfr.gov/cgi-bin/text-idx?SID=ae1deecc79a9f185d48af015ae277f5d&mc=true&tpl=/ecfrbrowse/Title21/21cfr1271_main_02.tpl)
- Tissue Guidances – General List
  - <https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/tissue-guidances>

# Contact Information

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- **OTAT Learn Webinar Series:**  
<http://www.fda.gov/BiologicsBloodVaccines>
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