

Risk of Bovine Spongiform Encephalopathy (BSE) in Collagenase Enzymes

Liberase HI (Roche Diagnostics Corporation, Indianapolis, IN) is a critical ancillary material used in the manufacture of human pancreatic islets for treatment of type 1 diabetes and chronic pancreatitis. Liberase HI is a mixture of purified enzymes with collagenase and protease activities specially formulated for rapid dissociation of human pancreas tissue and maximal recovery of intact, functional islets. Liberase HI has become the preferred collagenase used in human islet isolation. The specialized enzyme blend, commercially available beginning in 1995, is not manufactured under US current Good Manufacturing Practices (cGMPs), and therefore has always been labeled "For Research Use Only".

In April 2007, the FDA sent letters to all sponsors of Investigational New Drug (IND) applications in islet transplantation notifying them of the potential risk of introduction of transmissible spongiform encephalopathy (TSE) agents into the manufacturing process for islets. Specifically, there is a small risk of bovine spongiform encephalopathy (BSE) prions being present in Roche Liberase. Liberase is purified from culture supernatants of *Clostridium histolyticum* grown in medium containing Brain Heart Infusion (BHI) broth. The BHI is made from bovine brain and porcine heart. Bovine brain is considered "specified risk material" for use in human and animal foods and pharmaceuticals, based on the high infectivity of BSE-infected brain tissue. However, the level of risk varies depending on the age and geographic location of the source animals, the processing of the animal material before use, and the amount of downstream processing of the final food or pharmaceutical product.

Independently Roche Diagnostics Corp., has determined that the risk of BSE prions being present in the bovine material used in the production of BHI, carried through the production of the Liberase Purified Enzyme Blends and subsequent isolation and purification of cells used in clinical applications is remote, less than 1 in 1 million probability, based on the following information:

1. The bovine materials are believed (due to incomplete records received from a supplier) to have been sourced in the U.S, from livestock that are inspected and certified as fit for human consumption prior to slaughter. The incidence of BSE disease in such animals is negligible.
2. Several of the steps in the manufacturing of BHI and Liberase are expected, although not

validated, to significantly reduce the titer of infectious prions, including:

- extensive heat treatment and denaturation of BHI
 - prolonged exposure in fermentation cultures to a wide variety of potent proteases produced by *Clostridium histolyticum*
 - purification of Liberase enzymes by chromatography steps likely to reduce prion titers by at least 3 logs
3. In addition, Liberase is used early in the islet manufacturing process, with downstream washes and media changes providing additional reduction of potential prion titers.

IND sponsors are required to submit an amendment to FDA within 30 days of issuance of the letter. The amendment must include the following information:

1. whether Liberase, or any other collagenase manufactured with bovine brain, is utilized in processing of islets
2. whether informed consent materials adequately address the risk of BSE
3. revised consent forms and investigator brochures
4. intent and rationale for continued use of Liberase or other collagenases manufactured using bovine brain and the potential time frame of continued use
5. detailed information about each source of collagenase intended to be used
 - whether bovine material is used in manufacture
 - identity of any bovine materials used
 - animal sourcing information such as which country animals are born, raised and slaughtered in, age of cattle at slaughter, regulatory inspection of herds prior to slaughter
 - any additional evidence that source animals have not ingested animal feed prohibited under 21 CFR 589.2000 (e.g., retention of invoices and labeling of feed containing mammalian materials, certified compliance of feed manufacturers)

In addition, sponsors or investigators must obtain informed consent of study participants that is adequate under 21 CFR Part 50 and notify the appropriate Institutional Review Board (IRB). Consideration must also be given to prior collagenase sources no longer in use and the need to re-consent past study participants.

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Roche has temporarily placed a hold on the sale of Liberase to any institution they know has an IND including the Liberase Purified Enzyme Blends or Collagenase products. Roche hopes to release the hold on the sale of these products by summer 2007 once they can provide the required information on animal sources (PER FDA). Roche is changing the suppliers in its supply of BHI to ensure accurate and consistent information. In addition, Roche is pursuing development of a GMP-grade of Liberase that is free of any animal raw materials. This product is expected to be commercially available in 2008 although questions remain about whether an animal-free product can be produced that is as effective as the current product.

Alternate sources of collagenase are available, including a GMP-grade collagenase (Serva Electrophoresis, Heidelberg). The Serva collagenase is also manufactured with animal products, but no specified risk material is used. In addition, the safety of raw materials of animal origin has been certified by the European Directorate for the Quality of Medicines (EDQM). However, the Serva collagenase is significantly more expensive, requires blending with neutral protease and qualification studies may not be feasible by or available to all facilities. The clinical comparability of islets manufactured using alternate collagenases has not been well established.

ISCT will continue to monitor this situation and provide updated information as necessary. If the implications for islet manufacturers become onerous, ISCT may be in a position to mediate stakeholder meetings with FDA and Roche. Contact Lynn O'Donnell, Ph.D. at 614-293-9317 or email lynn.odonnell@osumc.edu for more information or concerns.

Collagenase enzymes are also used in manufacturing of other cell and tissue products to dissociate tissues, for example tumor dissociation. Roche has suspended sale of other collagenase products to IND holders in other applications as well. FDA has also issued letters to IND sponsors where they could identify collagenase use.

If a collagenase is used in manufacture of a cell therapy product, IND sponsors should determine if high-risk bovine materials, such as brain heart infusion media, are used in the manufacture of the collagenase. It may also be diligent practice to verify the presence of high-risk materials in any ancillary material not approved for human use that is isolated from bacteria that may be cultured in brain heart infusion media.

On January 12, 2007, FDA issued a proposed rule on the use of cattle materials in human drugs, "Proposed Rule: Use of materials Derived from Cattle in Medical products Intended for Use in Humans and Drugs Intended for Use in Ruminants," Federal Register 72(8) 1581-1619.

<http://www.fda.gov/cber/rules/catruminant.htm>