



**FOOD AND DRUG ADMINISTRATION**  
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

---

MEMORANDUM

DATE: 31-Jan-07 [REDACTED]

FROM: Paul Aebersold, Ph.D., Clinical Review Branch, HFM-392

SUBJECT: STN 125248, ZymoGenetics Recombinant Thrombin, Filing

TO: Mark Shields, Regulatory Project Manager, HFM-380

THROUGH: Toby Silverman, M.D., Chief, Clinical Review Branch, HFM-392

---

**Recommendation:**

With respect to the clinical section, this BLA can be filed. The Phase 3 study report contains electronic datasets accessible with JMP.

**Deficiencies Identified:**

A key review issue for a recombinant thrombin (r-thrombin) product is immunogenicity. As part of the Phase 3 clinical trial, blood samples were collected at baseline and at day 29. For subjects randomized to r-thrombin, the samples were analyzed by ELISA for antibodies to r-thrombin, CHO host-cell protein, and pro-thrombin activator. For subjects randomized to bovine thrombin, the samples were analyzed by ELISA for antibodies to bovine thrombin.

Sample analysis was done in three tiers. In the screening Tier 1, a single dilution of each sample was incubated in a well of the ELISA plate. The cut-off absorbance value for a positive screening result was chosen such that 90-95% of samples from untreated normal volunteers would be negative, i.e., 5-10% of samples would be presumed to be false positives. This assumption is flawed; given that a number of subjects entered into the trial had true positive baseline values, it could be assumed that some of the normal volunteers would also have true positive values. ***The validation report does not present data on the selection of the cut-off screening absorbance value.*** True positives, if any, should have been eliminated for purposes of selecting the screening cut-off absorbance, although they would have had little influence if they represented substantially less than 5% of the samples.

In the ELISA procedures, the "recommended" test sample dilution for Tier 1 was 1:50. In the Tier 2 analysis of positive Tier 1 samples, sample dilutions were 1:50, 1:150, 1:450, and 1:1350; titer was determined as the interpolated sample dilution whose absorbance was at the cut-point. ***It would thus appear that samples with antibody titers less than 50 would not be reported as positive. It is recommended that the sponsor repeat the assays using an initial dilution of 1:10 or 1:20.*** While the data do show that fewer subjects in the test group developed high-titer antibodies than in the control group, the consequences of developing low-titer antibodies to r-Thrombin are unknown. There could be very different implications for repeat exposure depending on whether a patient developed no antibodies or developed low-titer antibodies after first exposure.

The validation report does not assess relative sensitivities of the ELISAs for antibodies to bovine or recombinant thrombin. It appears from the procedures that plates for both ELISAs were coated with [REDACTED] of antigen, but without comment as to relative specific activities of the two thrombins. Non-specific background samples were different: [REDACTED] for the recombinant thrombin ELISA, [REDACTED] for the bovine thrombin ELISA. Positive antibody controls were raised in [REDACTED] antibodies to bovine thrombin provided higher absorbance signals at lower concentrations than [REDACTED] antibodies to recombinant thrombin. ***It is recommended that data be provided for each ELISA that demonstrate the plateau of absorbance.*** It could well be that the titers are higher for [REDACTED] anti-bovine than for [REDACTED] anti-recombinant; but if the absorbances at plateau are different, it could also be that antigen coatings for the two ELISAs are different, leading to differing sensitivities.

In the validation assays for titer, [REDACTED] anti-bovine thrombin antibody provided a reliable signal above the cut-off value, whereas it took [REDACTED] of [REDACTED] anti-recombinant thrombin antibody to provide an equal robust signal. This illustrates why it is important to determine whether the assays are not equally sensitive or whether the [REDACTED] positive control antibodies simply do not have equal titers.