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Validating a faster method for reconstitution of Crotalidae Polyvalent Immune Fab (ovine)

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ABSTRACT

Background: Reconstitution of CroFab® (Crotalidae Polyvalent Immune Fab [ovine]) lyophilized drug product was previously performed using 10 mL sterile water for injection followed by up to 36 min of gentle swirling of the vial. CroFab has been clinically demonstrated to be most effective when administered within 6 h of snake envenomation, and improved clinical outcomes are correlated with quicker timing of administration. An alternate reconstitution method was devised, using 18 mL 0.9% saline with manual inversion, with the goal of shortening reconstitution time while maintaining a high quality, efficacious product.

Methods: An analytical study was designed to compare the physicochemical properties of 3 separate batches of CroFab when reconstituted using the standard procedure (10 mL WFI with gentle swirling) and a modified rapid procedure using 18 mL 0.9% saline and manual inversion. The physical and chemical characteristics of the same 3 batches were assessed using various analytic methodologies associated with routine quality control release testing. In addition further analytical methodologies were applied in order to elucidate possible structural changes that may be induced by the changed reconstitution procedure.

Results: Batches A, B, and C required mean reconstitution times of 25 min 51 s using the label method and 3 min 07 s (a 88.0% mean decrease) using the modified method. Physicochemical characteristics (color and clarity, pH, purity, protein content, potency) were found to be highly comparable. Characterization assays (dynamic light scattering, analytical ultracentrifugation, LC–MS, SDS-PAGE and circular dichroism spectroscopy were also all found to be comparable between methods.

Discussion: When comparing CroFab batches that were reconstituted using the labeled and modified methods, the physicochemical and biological (potency) characteristics of CroFab were not significantly changed when challenged by the various standard analytical methodologies applied in routine quality control analysis. Additionally, no changes in the CroFab molecule regarding degradation, aggregation, purity, structure, or mass were observed.

Conclusion: The analyses performed validated the use of the more rapid reconstitution method using 18 mL 0.9% saline in order to allow a significantly reduced time to administration of CroFab to patients in need.

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1. Introduction

Approximately 4700 snakebites are reported to poison control centers annually in the United States (Spiller et al., 2010) and the majority of envenomations in the US are caused by North American pit viper bites (Gold et al., 2004). North American pit viper venoms can include procoagulants, anticoagulants, cardiotoxins, hemotoxins, and neurotoxins (Chippaux and Goyffon, 1998; Seifert and Boyer, 2001). Clinical manifestations of pit viper envenomation range from tissue necrosis to loss of joint function. Local sequelae of envenomation can include partial or complete loss of digits, loss of function at a joint and permanent sensory loss (Dart et al., 1996; Hill et al., 2001). Snake venom can linger in tissue as a depot of venom often remains in the body after envenomation, which may prolong its clinical effects (Seifert and Boyer, 2001; Boyer et al., 2001). Outcomes vary and effects can range from nothing apparent clinically to a life-threatening condition, and clinical signs of envenomation often become evident within minutes of the bite. Tissue damage in particular is time-dependent and the use of lyophilized Crotalidae Polyvalent Immune Fab (ovine) (BTG International Inc., 2012) is recommended within 6 h of the bite (Dart and McNally, 2001).

Each vial of CroFab contains approximately 1 g of total protein and was originally labeled to be reconstituted with 10 mL of sterile water for injection USP (WFI) and gently swirled for approximately 20–25 min until dissolution (BTG International Inc., 2012). The dissolved vials are then further diluted in normal saline prior to intravenous infusion.

CroFab reconstitution time was challenged in an experiment (Quan et al., 2010) that concluded that reconstitution time could be improved through an increase in solvent volume (up to 25 mL of WFI), and through the use of inversion mixing. While such changes may improve dissolution time, other effects that could impact the quality, safety, and efficacy of CroFab could potentially occur. The purpose of the present study was to confirm the feasibility and impact on product quality of an alternative more rapid method of reconstitution, in order to reduce the overall time from snakebite envenomation to administration of CroFab.

2. Materials and methods

This study was carried out to assess the impact on reconstitution time and product quality attributes of applying a vial reconstitution method that uses 18 mL of 0.9% (w/v) normal saline with manual inversion in comparison to the labeled method (10 mL WFI with gentle swirling until complete dissolution; a process which may take up to 36 min). Various attributes of CroFab drug product material were assessed including physicochemical properties (pH, color, clarity, mass and concentration), protein aggregation, protein conformation, purity, and product potency to assess comparability between reconstitution procedures.

The study appraised 3 different CroFab batches reconstituted according to the labeled method, and sample vials from the same 3 batches reconstituted using the proposed alternative method (see Fig. 1). The analytical procedures

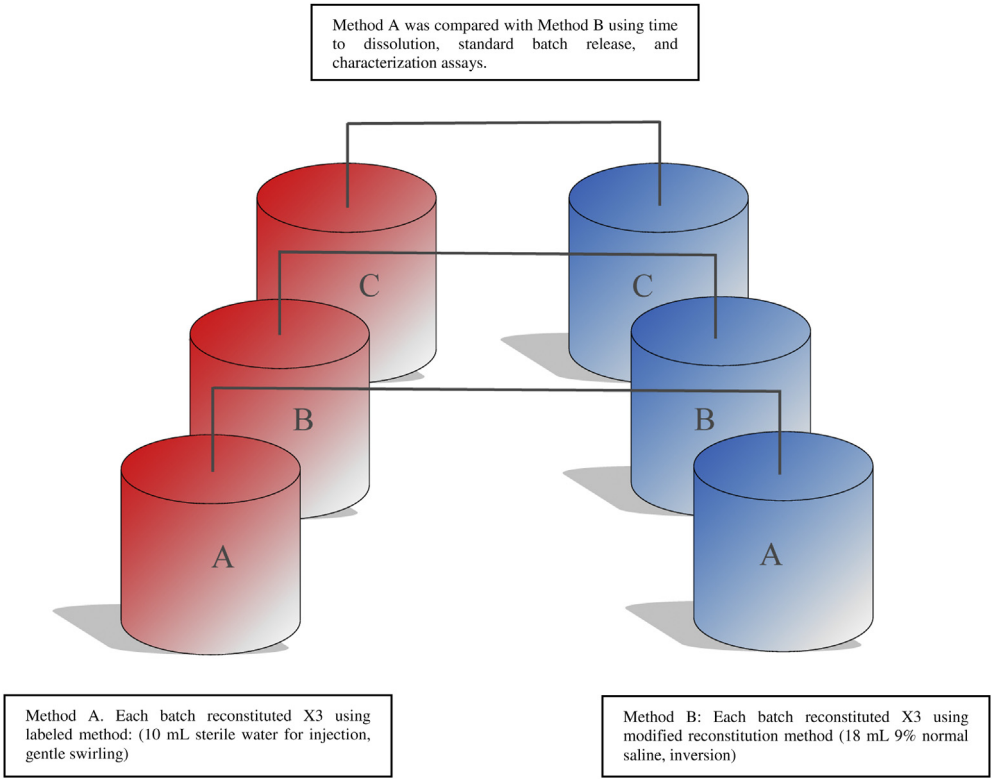

Fig. 1. Reconstitution method study schematic.