Manufacturing Process of Intratect efficaciously eliminates thrombogenic potential

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Abstract

In 2010, an unexpected increase in the reporting rate of serious thromboembolic adverse events associated with the application of immunoglobulin preparations from one specific manufacturer caused increased concern regarding this topic. As a result, monographs for immunoglobulin preparations were revised. Hence, plasma manufacturers were required to validate at least one step in the manufacturing process for the effective reduction of the thrombogenic potential in immunoglobulin preparations. Biotest validated the step in their manufacturing process of their immunoglobulin products which incorporates caprylic acid treatment and performed detailed analyses including non-activated partial thromboplastin time, thrombin generation assay, factor Xla levels, total proteolytic activity and prekallikrein levels at various stages in the production process.

Biotest demonstrated that treatment with caprylic acid during manufacturing was effective in reducing the thrombogenic potential resulting in the generation of an immunoglobulin product with an improved safety profile. Moreover, Biotest showed the absence of various factors involved in the coagulation cascade in all of the Biotest immunoglobulin products. Through producing high quality immunoglobulin products, Biotest is committed to ensuring maximum patient safety.

Introduction

Thromboembolic events (TEEs), in the context of temporary increased viscosity, are an established class-related risk associated with immunoglobulin treatment. TEEs are usually associated with certain risk factors, including advanced age, infusion volume and rate and patient’s medical history of vascular disease or thrombotic episodes (EMA Guidelines 2012). In 2010, an unexpected increase in the reporting rate of serious TEEs with the use of intravenous immunoglobulin (IVIG) preparations from one specific manufacturer highlighted the risk associated with immunoglobulins as a class.

The main factors contributing to TEEs are the presence of procoagulant substances, particularly factor Xla which was identified as the probable primary cause of these incidents (Roemisch et al., 2011; Etscheid et al., 2012). Moreover, in a preparation for subcutaneous administration, a high concentration of prekallikrein/kallikrein was observed (Schulte, 2011).

As a result, the monographs on "Human normal immunoglobulin for intravenous administration (0918)" and "Human normal immunoglobulin (0338)" were revised by regulatory authorities. Subsequently, all manufacturers producing immunoglobulin products from human plasma were required to demonstrate that certain manufacturing steps were in place capable of removing thrombosis-generating agents. It also had to be shown that the final immunoglobulin product "does not exhibit thrombogenic (procoagulant) activity" (EMA monograph 0918).

Therefore, Biotest carried out a thorough investigation of its manufacturing processes of IVIGs and subcutaneous immunoglobulins with regard to the thrombogenic potential of these products. Specifically, Biotest investigated non-activated partial thromboplastin time (NaPTT), thrombin generation assay (TGA), factor Xla activity, total proteolytic activity (PA) and prekallikrein (PKK) levels at various production steps, and a variety of additional coagulation factors in the final drug product. In order to comply with the regulatory request for validation of one manufacturing step for the reduction of thrombogenic substances, Biotest choose to validate the most capable process step which incorporates caprylic acid treatment in order to reduce thrombogenic substances. Furthermore, Biotest tested for the absence of various components of the coagulation cascade (factors VIII, IX, X, XI, XII, and the activated factors IIa, IXa, Xa) in all Biotest immunoglobulin products in order to determine the safety of these products.

In general, Biotest incorporates several approaches to ensure production of efficacious products with favorable safety profiles, in turn providing maximal patient safety. As for all products from Biotest, quality of their immunoglobulin products is based on a number of parameters. These include aspects regarding process understanding, thorough performance of production and quality, and continuous pharmacovigilance during the pre- and
post-authorization phase of any product (illustration 1). Through full understanding of the process, the manufacturing steps were designed so that quality can be permanently assured. As a result of the revision of the monograph, a detailed risk analysis was performed and every process step was evaluated with regard to the potential generation or reduction of thrombogenic substances by activation/concentration of clotting factors, or inactivation/removal of clotting factors.

With two major production steps (fractionation of fraction I/III and treatment with caprylic acid) for the reduction of thrombogenic substances, and continuous process controls, Biotest has incorporated stringent measures for manufacturing a safe immunoglobulin product. The incorporation of these two steps for effective reduction of thrombogenic substances illustrates the commitment of Biotest to the quality of its products and hence patient’s safety which is of major importance to the company.

As well as reducing the thrombogenic potential at certain steps in the manufacturing process, to test the safety of Biotest’s IVIG products, further measurements regarding the levels of thrombogenic substances in the final drug product were performed. Finally, Biotest’s pharmacovigilance activities ensure that the safety of the therapeutic use of the product is continuously and thoroughly monitored so that an unexpected signal, even if very rare, would be reliably detected and managed. Monitoring includes collection, documentation, evaluation and assessment of suspected adverse drug reactions and quality complaints originating from all sources worldwide and throughout the life-cycle of a product.

Methods

Materials

For reference purposes a serum pool was prepared from at least 20 donors to eliminate individual assay variability which could occur, as has been described previously (Roemisch et al., 2012). This serum pool served as a positive control for the NaPTT, TGA and FXIa assays and was stored at ≤ -70°C within 8 hours of blood sampling. For each batch of serum pool, assay specific target tolerance ranges were determined from at least 6 mutually independent measurements.

Analytical Methods

Non-activated partial thromboplastin time (NaPTT) measurement

The NaPTT of immunoglobulins was performed on the basis of European Pharmacopoeia 2.6.22 with an adaptation to 1:4 dilution, in place of 1:10 and 1:100 dilutions, to increase sensitivity for thrombogenic substances possibly contained in immunoglobulins. In addition to the coagulation time in seconds, the normalized coagulation time (as related to the coagulation time of the test buffer) was reported as a percentage.

Thrombin generation assay (TGA)

Thrombin generation in samples was measured using a POLARstar Omega multidetection fluorescence polarization microplate reader (BMG LABTECH) using TGA reagents and calibrator manufactured by Technoclone GmbH. In brief, 60 µl substrate-RCH mixture and 30 µl deficient plasma were used per well. For the calibration series no deficient plasma was used. 40 µl of the corresponding calibrator or 10 µl of the corresponding sample were analyzed. If necessary (e.g. thrombin peak above calibration), a dilution of the sample in Tris-NaCl-Buffer with 0.5% BSA was performed. Each measurement was determined at least twice. Deficient plasma, e.g. FXI-deficient plasma for immunoglobulins, was used as the basis for determining the thromboembolic potential of immunoglobulins.

Factor Xla (FXIa) activity

FXIa activity in samples was determined using a chromogenic assay (BIOPHEN Factor Xla, A220412). The assay was performed as described by the manufacturer using a kinetic method in a coagulation analyzer. Control and samples were measured at least twice.

Prekallikrein (PKK) measurements

Plasma kallikrein activator (Unicorn 0071, containing ellagic acid, phospholipid and a plasma fraction with FXII and high molecular weight kininogen) converts prekallikrein to kallikrein. The resultant kallikrein activity can then be determined kinetically via proteolytic cleavage of the chromophor para-nitroaniline from the chromogenic substrate S-2302 (Chromogenix). The concentration increase of cleaved para-nitroaniline was determined at 405 nm over time which correlates with prekallikrein activity. The assay was performed using an automated kinetic method in a coagulation analyzer. Control and samples were measured at least twice. Normal Reference Plasma was used for calibration and Reference Control Normal as the positive control (both from Precision Biologics).

Total proteolytic activity (PA)

For the assessment of total proteolytic activity,
samples were analyzed using a kinetic method with the chromogenic substrate S-2288 (Chromogenix). The change in absorption per minute is directly converted into U/l as described by the manufacturer of the chromogenic substrate (Chromogenix).

**Factor IIa (FIIa), Factor Xa (FXa) and Factor IXa (FIXa) activity**

Activity of activated coagulation factors IIa and Xa was determined using an automated kinetic chromogenic method with S-2238 (FIIa) or S-2765 (FXa) as the chromogenic substrate in a coagulation analyzer. Purified factors IIa (Enzyme Research) or Xa (Chromogenix) were used for calibration. Activity of factor IXa was determined using the BIOPHEN Factor IXa test kit (Hyphen BioMed) adapted to an automated kinetic method.

**Factor VIII (FVIII), Factor IX (FIX), Factor X (FX), Factor XI (FXI) and Factor XII (FXII) activity**

The activity of coagulation factors VIII, IX, X, XI and XII was determined using an automated kinetic clotting method with Actin FSL (FVIII, FIX, FXI, FXII) or Innovin (FX) as an activator (Siemens) and the respective deficient plasmas in a coagulation analyzer. Standard human plasma (Siemens) was used for calibration.

### Pharmacovigilance

At Biotest, product safety is of central importance. High product quality is complemented by comprehensive pharmacovigilance procedures throughout the development of a medicinal product, from early-phase clinical trials through to post-authorization and until the end of a product's life cycle. During the latter, safety data spontaneously reported from sources such as healthcare professionals and/or patients, as well as comprehensive real-life safety data from non-interventional studies are collected.

Following the observed issue on thromboembolic events (TEEs), EU marketing authorization holders of immunoglobulin preparations were requested to perform an evaluation of the TEE status for the time period January 2001 to December 31st, 2011.

At Biotest, the evaluation was performed for the intravenous (iv) immunoglobulin Intratect and the subsequent iv "FH" family, including Cytotect CP (FH), Hepatect CP (FH), Varitect CP (FH), and the immunoglobulins for subcutaneous (sc) administration, Zutebra and Fovepta.

The Biotest global pharmacovigilance database was searched for TEEs from all sources (all reporters), by every category (spontaneous reporting, clinical trials and non-interventional studies) using the Standard MedDRA Query (SMQ) ‘Embolic and thrombotic events’. Cases identified as a result of this search were re-evaluated manually by expert health care professionals experienced in pharmacovigilance.

### Results

**Validation of the process step "Treatment with caprylic acid"**

A major requirement of the regulatory authorities was the validation of one production step capable of reducing thrombogenic substances during the manufacture of immunoglobulin products. For this validation, the production step using treatment with caprylic acid was chosen since this is the most effective step for the reduction of thrombogenic substances. During this manufacturing step, the effectiveness of different amounts of caprylic acid (standard, low and very low) and different pHs (standard, low or high) in reducing thrombogenic substances, were tested. Samples were analyzed according to the above described methods (TGA, NaPTT, Factor XII, PA and PKK) before and after treatment with caprylic acid. Results are shown in illustrations 2-5.

**Measurement of Biotest drug product: Intratect**

Further evidence regarding the safety of the product Intratect was gained by detailed measurement of various parameters with thrombogenic potential in this product. It could be shown, that all evaluated parameters were below the limit of quantification for Intratect (illustrations 6 and 7). Thus a reduction of thrombogenic substances throughout the production process was achieved, conferring an improved safety profile for this product.

**Measurement of Biotest drug products: All immunoglobulin products**

In order to assess the safety of all of Biotest’s immunoglobulin products, these were evaluated using standard test methods and the NaPTT and TGA analyses. It could be shown that in all preparations, a reduction of thrombogenic substances was achieved (illustrations 8 and 9).

**Pharmacovigilance**

For Cytotect CP (FH), Hepatect CP (FH), Varitect CP
(FH), Zutepta and Fovepta no TEE reports have been received during the reporting period (2001 to 2011).

For Intratect, a total of 21 reports on TEEs have been received during that time period; whereas 14 out of these 21 reports originated from a non-interventional observational study (NIS), one (1) report originated from a clinical trial, and 6 reports were obtained from spontaneous reporting.

Notably, during the Biotest NIS, an advanced adverse event reporting scheme, comparable to that used in interventional clinical trials, was performed. All adverse events (AEs) were collected and documented independent from a causal relationship and on this basis adverse drug reactions (ADRs) were identified based on medical assessment: 9 out of the 14 AEs from the NIS were assessed as "not related" and 2 (of 14) were assessed as "unlikely related" to Intratect by the reporting physician and also by the company. The remaining 3 (of 14) AEs were regarded as suspected ADRs (at least possibly related).

The 1 report received from a clinical trial (study number 960) was assessed as causally 'not related' to Intratect by the investigator and also by the company.

For the 6 unsolicited spontaneous reports, the principle of implied causality was applied. These reports correspond cumulatively to approximately 340,000 Defined Single Standard Doses of Intratect (30 g protein) respective infusions.

Of the 6 spontaneous TEE reports for Intratect, 2 events occurred in 2008, 1 event in 2010, and 3 events in 2011. In all other calendar years, no TEE reports were received for Intratect. The reporting rate per year relative to exposure, derived from the spontaneous reporting is as follows:

1. 2008 (2 spontaneous reports):
   1 report per 739,360 g protein distributed, or
   1 report per 24,645 Defined Single Standard Doses
2. 2010 (1 spontaneous report):
   1 report per 1,960,635 g protein distributed, or
   1 report per 65,355 Defined Single Standard Doses
3. 2011 (3 spontaneous reports):
   1 report per 803,362 g protein distributed, or
   1 report per 26,779 Defined Single Standard Doses
4. 2001 – 2007; 2009:
   Reporting rate= 0

In conclusion, for the period evaluated (January 2001 to 31 December 2011) there were no findings indicating an unexpected increase in thromboembolic events (TEEs) with the Biotest immunoglobulin FH product family, either in terms of severity, nature, frequency or patient population at risk.

Discussion

Particularly when unexpected in terms of nature, frequency or severity, suspected adverse drug reactions (adverse events considered causally related to medicinal products) are always of concern to all stakeholders in the healthcare system, and especially for patients and prescribers. As a result of an investigation into an increased frequency of thromboembolic adverse events with an immunoglobulin preparation from one manufacturer, root cause analysis revealed occasionally increased concentrations of activated FXI, correlating with elevated levels measured in the TGA (Roemisch et al., 2011) and increased levels of prekallikrein/ kallikrein (Etscheid et al., 2012). As a result, regulatory authorities revised the monographs for immunoglobulin products (EMA monographs 0918 "Human normal immunoglobulin for intravenous administration" and 0338 "Human normal immunoglobulin") insofar that all manufacturers of plasma derived products were required to investigate both end-product and production processes carefully in relation to thrombogenic substances.

To comply with implementation of the revised monographs, Biotest conducted a thorough and detailed analysis of its overall production process, including validation of one production step in relation to the reduction of thrombogenic substances. Caprylic acid treatment is well known for eliminating impurities and also potentially thrombogenic substances (Morais and Massaldi, 2012) and on the basis of this, Biotest chose this process step for validation. In the laboratory the process was validated using both standard parameters and sub-optimal conditions, identified as low amount of caprylic acid added per kg solution and a high pH value. Fraction II from an intermediate supplier was also included in the investigations for comparison and experimental control. Analyses included the TGA, NaPTT and PA tests for general assessment of the occurrence of thrombogenic substances both in samples before and after caprylic acid treatment. Factor XIa and prekallikrein were tested to specifically address the root cause of biochemical parameters leading to TEEs in samples before and after caprylic acid treatment and batches of immunoglobulin product (Intratect).
In this study it could be shown that all parameters investigated were substantially reduced in terms of thrombogenic activity at the production step using caprylic acid. A reduction of all thrombogenic substances measured, was accomplished by the treatment with caprylic acid, even when using sub-optimal conditions (low amount of caprylic acid/high pH). Therefore this step proved to be effective in generating a safer immunoglobulin product. Hence it could be shown in detail that thrombogenic substances are reduced by this validated production step, as required by authorities in the fulfillment of the change to the monographs for immunoglobulin products.

These data were further supported by measurements of the various coagulation and activated coagulation factors (factors VIII, IX, X, XI, XII, and the activated factors IIa, IXa, Xa) in the drug product Intratect at different concentrations (50 g/l and 100 g/l). At both concentrations, all measurements were below the limit of quantification (illustrations 6 and 7), confirming the quality of the immunoglobulin product in terms of thrombogenic substances.

Investigations of other products in the Intratect family and other Biotest immunoglobulin products, Cytotect CP, Hepatect CP, Varitect CP and Zutectra, showed that no critical levels of thrombogenic substances were present as measured using the global tests of NaPTT and TGA.

A comprehensive and detailed analysis of pharmacovigilance data did not indicate an increased risk of TEEs with the Biotest FH IVIG product family. No batch-related or time-dependent cluster has been identified, nor any new information exceeding existing class related warnings and precautions on TEEs with regard to frequency, patient population and risk factors. Overall, the Biotest “FH” product family has a well-established benefit-risk profile. The presented data do not provide any further evidence of previously unknown risks or safety issues with regard to TEEs. Therefore, Biotest has demonstrated that an effective production process is in place for the manufacture of their immunoglobulin products with a validated step for reducing thrombogenic potential resulting in safer products. Together, with an overall understanding of the processes involved from production, continuous quality control, in-depth analytical knowledge and post marketing surveillance, Biotest is able to ensure the continuous high quality and efficacy of its immunoglobulin products for optimal treatment of patients.

Abbreviations

ADR: Adverse drug reaction
AE: Adverse event
EMA: European Medicines Agency
F: Coagulation factor
IVIG: Intravenous immunoglobulin
NaPTT: Non-activated partial thromboplastin time
NIS: Non-interventional observational study
PA: Total proteolytic activity
PKK: Prekallikrein
TEE: Thromboembolic event
TGA: Thrombin generation assay

References

8. Roemisch JR, Kaar W, Zoechling A, Kannicht C,

Illustrations

Illustration 1

Quality by design driven approach to gain high quality and safe IgG products.
Illustration 2

TGA before and after treatment with caprylic acid (CA). Peak thrombin levels (%) for samples, compared to Normal Reference Plasma, are shown. 1, 2: Standard amount of CA, standard pH; 3: Low amount of CA, high pH; 4: Standard amount of CA, standard pH; 5: Standard amount of CA, high pH; 6: Low amount of CA, standard pH; 7: Low amount of CA, high pH; 8: Very low amount of CA, standard pH; 9: Very low amount of CA, high pH.
### Illustration 3

Measurement of samples with the TGA before and after treatment with caprylic acid (CA). Peak thrombin levels (nM) are shown. Starting material from different sources (intermediate supplier; Biotest) and various combinations of relevant parameters (amount caprylic acid and pH) were evaluated. SD, ± standard deviation.

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Experimental parameter</th>
<th>pH-value after caprylic acid treatment</th>
<th>TGA Thrombin [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount caprylic acid [g/kg] and pH</td>
<td></td>
<td>Before caprylic acid treatment</td>
</tr>
<tr>
<td>Fraction II from supplier</td>
<td>Standard amount CA/kg solution; standard pH</td>
<td>5.5</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>Standard amount CA/kg solution; standard pH</td>
<td>5.5</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>Low amount CA/kg solution; high pH</td>
<td>5.6</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td>Fraction II from Biotest</td>
<td>Standard amount CA/kg solution; standard pH</td>
<td>5.5</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>Standard amount CA/kg solution; high pH</td>
<td>5.6</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>Low amount CA/kg solution; standard pH</td>
<td>5.5</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>Low amount CA/kg solution; high pH</td>
<td>5.6</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>Very low amount CA/kg solution; standard pH</td>
<td>5.5</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>Very low amount CA/kg solution; high pH</td>
<td>5.6</td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
</tbody>
</table>
Illustration 4

NaPTT before and after treatment with caprylic acid (CA). The percentage of the value compared to the buffer blank is shown. 1, 2: Standard amount CA, standard pH; 3: Low amount CA, high pH; 4: Standard amount CA, standard pH; 5: Standard amount CA, high pH; 6: Low amount CA, standard pH; 7: Low amount CA, high pH; 8: Very low amount CA, standard pH; 9: Very low amount CA, high pH.

*Values > 80% are regarded as uncritical as suggested as cut-off by Funk et al., 2013.
Illustration 5

Measurement of samples for factor Xla (FXIa), prekallikrein (PKK) and proteolytic activity (PA) before and after treatment with caprylic acid (CA). Peak thrombin levels (nM) are shown. Starting material from different origin and various combinations of relevant parameters (amount caprylic acid and pH) were evaluated. SD, ± standard deviation.

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Experimental parameter</th>
<th>FXIa</th>
<th>PKK</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng/ml</td>
<td>% of Norm</td>
<td>U/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>before caprylic acid treat-ment</td>
<td>after caprylic acid treat-ment</td>
<td>before caprylic acid treat-ment</td>
</tr>
<tr>
<td>Fraction II from supplier</td>
<td>Standard amount CA/kg solution; standard pH</td>
<td>Average 161.9</td>
<td>0.3</td>
<td>34.2</td>
</tr>
<tr>
<td>SD</td>
<td>14.14</td>
<td>0.07</td>
<td>0.28</td>
<td>n. d.*</td>
</tr>
<tr>
<td>Standard amount CA/kg solution; standard pH</td>
<td>Average 142.5</td>
<td>0.3</td>
<td>35.8</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>SD</td>
<td>8.06</td>
<td>0.02</td>
<td>1.63</td>
<td>n. d.*</td>
</tr>
<tr>
<td>Low amount CA/kg solution; high pH</td>
<td>Average 211.6</td>
<td>1.0</td>
<td>26.4</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>SD</td>
<td>4.81</td>
<td>0.18</td>
<td>1.63</td>
<td>n. d.*</td>
</tr>
<tr>
<td>Fraction II from Biotest</td>
<td>Standard amount CA/kg solution; standard pH</td>
<td>Average 0.4</td>
<td>0.2</td>
<td>2.9</td>
</tr>
<tr>
<td>SD</td>
<td>0.49</td>
<td>0.04</td>
<td>0.00</td>
<td>n. d.*</td>
</tr>
<tr>
<td>Standard amount CA/kg solution; high pH</td>
<td>Average 0.4</td>
<td>0.2</td>
<td>3.1</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.44</td>
<td>0.01</td>
<td>0.21</td>
<td>n. d.*</td>
</tr>
<tr>
<td>Low amount CA/kg solution; standard pH</td>
<td>Average 0.8</td>
<td>0.3</td>
<td>3.3</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.18</td>
<td>0.06</td>
<td>0.07</td>
<td>n. d.*</td>
</tr>
<tr>
<td>Low amount CA/kg solution; high pH</td>
<td>Average 0.5</td>
<td>0.3</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.57</td>
<td>0.07</td>
<td>n. d.</td>
<td>n. d.*</td>
</tr>
<tr>
<td>Very low amount CA/kg solution; standard pH</td>
<td>Average 0.9</td>
<td>0.5</td>
<td>&lt;2.5</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.26</td>
<td>0.10</td>
<td>n. d.</td>
<td>n. d.*</td>
</tr>
<tr>
<td>Very low amount CA/kg solution; high pH</td>
<td>Average 0.4</td>
<td>0.4</td>
<td>3.3</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.44</td>
<td>0.14</td>
<td>0.07</td>
<td>n. d.*</td>
</tr>
</tbody>
</table>

*n. d. – not determinable
Illustration 6

Analysis of coagulation factors present in: Intratect (50 g/l), Intratect (50 g/l) produced from fraction II of an intermediate supplier and Intratect (100 g/l).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Intratect (50 g/l)</th>
<th>Intratect (50 g/l) with fraction II from supplier</th>
<th>Intratect (100 g/l)</th>
<th>Mean / Median</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII</td>
<td>U/ml</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Factor IX</td>
<td>U/ml</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
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</tr>
<tr>
<td>Factor X</td>
<td>U/ml</td>
<td>&lt; 0.10</td>
<td>&lt; 0.10</td>
<td>&lt; 0.10</td>
<td>&lt; 0.10</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td>Factor XI</td>
<td>U/ml</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Factor XII</td>
<td>U/ml</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
Illustration 7

Analysis of activated coagulation factors in: Intratect (50 g/l), Intratect (50 g/l) produced from fraction II of an intermediate supplier and Intratect (100 g/l).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Intratect (50 g/l)</th>
<th>Intratect (50 g/l) with fraction II from supplier</th>
<th>Intratect (100 g/l)</th>
<th>Mean / Median</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor IIa</td>
<td>IU/ml</td>
<td>&lt; 0.063</td>
<td>&lt; 0.063</td>
<td>&lt; 0.063</td>
<td>&lt; 0.063</td>
<td>&lt; 0.063</td>
</tr>
<tr>
<td>Factor IXa</td>
<td>mU/ml</td>
<td>&lt; 0.2</td>
<td>&lt; 0.2</td>
<td>&lt; 0.2</td>
<td>&lt; 0.2</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Factor Xa</td>
<td>IU/ml</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
Illustration 8

Measurement of Biotest immunoglobulin (IVIG) products with the NaPTT assay. The critical level was set to 150 seconds.

NaPTT (1:4) in drug products

<table>
<thead>
<tr>
<th>Product</th>
<th>Cytotect CP</th>
<th>Hepatect CP</th>
<th>Intratect</th>
<th>Intratect 100g/l</th>
<th>Varitect CP</th>
<th>Zutectra</th>
</tr>
</thead>
</table>

NaPTT (1:4) [sec.]
Illustration 9

TGA measurement of Biotest immunoglobulin (IVIG) products. The critical level was set at 350 nM peak thrombin.