In-vitro characterisation of the first therapeutic factor V concentrate

Andrew S Lawrie[1], Chiara Berbenni[2], Marzia Menegatti[2], Ilaria Nardini[3], Ian J Mackie[1], Samuel J Machin[1], **Flora Peyvandi**[1,2,4]

[1] Haemostasis Research Unit, Department of Haematology, University College London, London, UK
[2] Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda , Ospedale Maggiore Policlinico, Milan, Italy
[3] Kedrion S.p.A - Loc. Ai Conti 55051 - Castelvecchio, Pascoli Barga (Lucca), Italia
[4] Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Università degli Studi di Milano, Milan, Italy

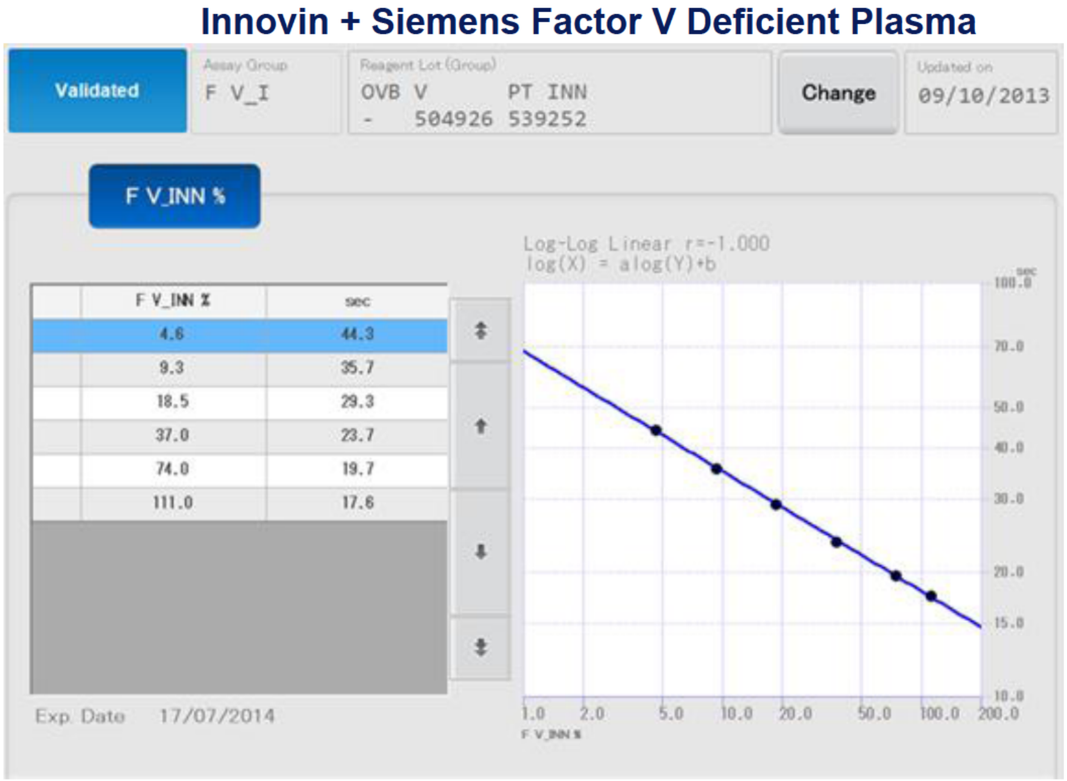
Background: Congenital factor V (FV) or combined FV & VIII deficiency can give rise to a life threatening bleeding disorder. The standard treatment for FV deficiency is fresh frozen plasma (FFP), which can cause problems with hypervolaemia, since with FFP the FV level can realistically only be elevated to approximatly 20% of normal in severely affected patients (i.e. FV<1%). Kedrion S.p.A is developing a plasma derived therapeutic factor V concentrate that has solvent-detergent treatment and nanofiltration steps within the manufacturing process.

Methods: To assess haemostatic efficacy of the concentrate, FV procoagulant activity (FV:C) and antigen (FV:Ag) levels were assayed.

- FV:C relative to the WHO International Standard for FV activity (03/116) using a range of thromboplastin reagents and FV depleted plasmas (Figure 1) in parallel-line bioassays system on Sysmex CS-5100 analyser (Figure 2)

Reagent Group 1	Reagent Group 2	Reagent Group 3	Reagent Group 4
Innovin / Siemens factor V deficient	Innovin / Precision Biological factor V deficient	PT Fib HS Plus / IL factor V deficient	Recombiplastin 2G / IL factor V deficient

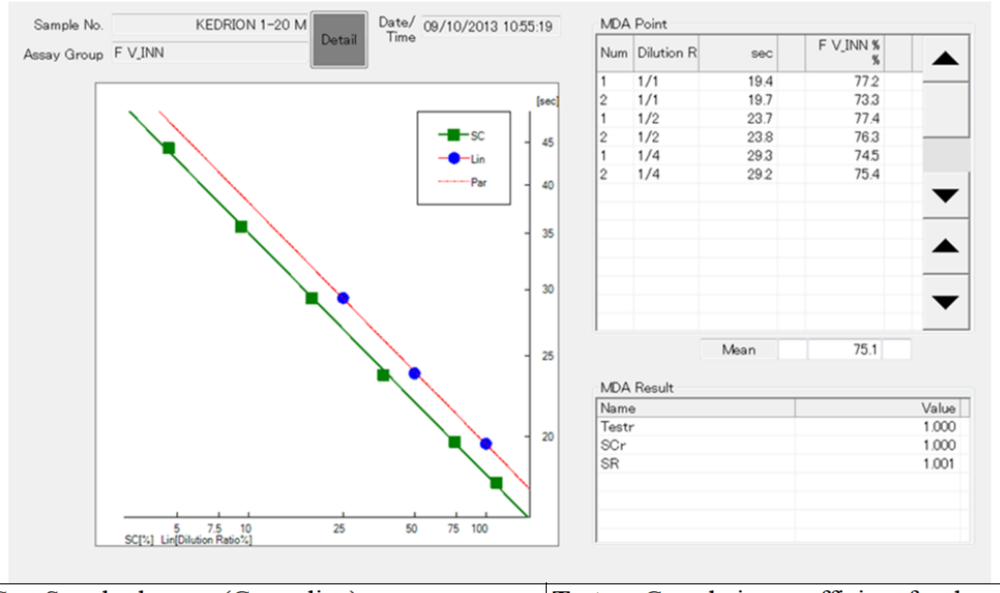
Figure 1. Example of a CS-5100 Calibration Curves using Innovin + Siemens Factor V Deficient Plasma



- On three consecutive days, concentrate was thawed at 37°C and then left for 30 minutes at ambient temperature to equilibrate.
- The FV concentrate was then pre-diluted in three sources of deficient plasma prior to determining the relative potency.
- This process was repeated at 2 hours post thawing of the FV concentrate.
- FV:Ag was measured using Zymutest Factor V (Hyphen BioMed).

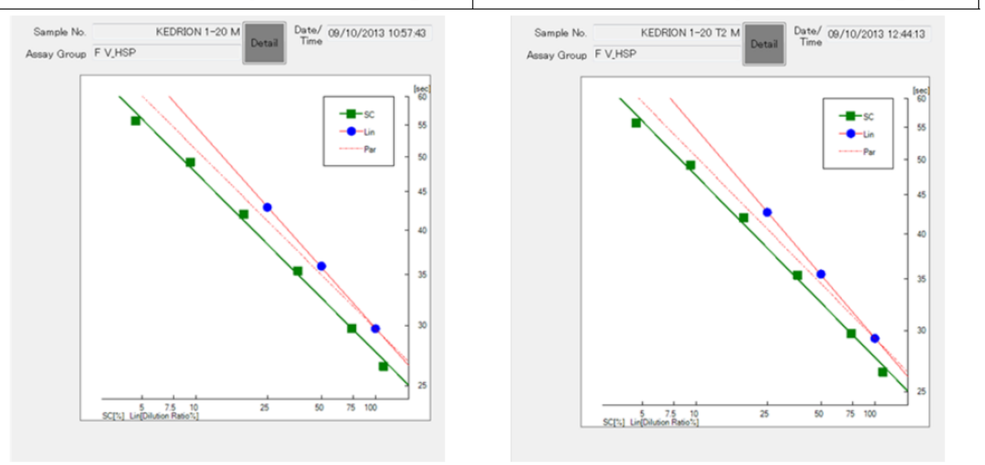
Results: As seen in Table 1 the four reagent combinations over the three days gave a mean relative potency at 30 minutes of: FV:C=14.8 IU/mL (CV=9.2%) and at 2 hours: FV:C=14.6 IU/mL (CV=10.4%), although some of the reagent combinations gave poor assay parallelism (Figure 2). Using the antigen kit reference preparation (the WHO standard is only calibrated for activity) a mean FV:Ag=16.1 U/mL (CV=9.3%) was obtained giving a FV:C/FV:Ag ratio of 92%.

Figure 2. CS-5100 Analyser evaluation of linearity and parallelism (using Innovin + Siemens Factor V Deficient Plasma)
(The left hand portion of this screen will be presented in the results section)



SC = Standard curve (Green line)	**Testr** = Correlation coefficient for the test sample
Lin = best straight line through test samples data points (solid red line)	**SCr** = Correlation coefficient for the standard curve
Par = line through the first test dilution data point parallel to the standard curve (broken red line)	**SR** = Calibrator / Test Slope Ratio (indication of parallelism)

Figure 3. Example of Standard / Concentrate Linearity and Parallelism using Fibrinogen HS Plus + IL Factor V Deficient Plasma



Calibrator / Test Slope Ratio = 1.125	**Calibrator / Test Slope Ratio = 1.152**

Parallelism was assessed by comparing the regression slope ratio for each test sample to that of the calibration curve (a normal slope ratio should be in the range 0.9-1.1)

Table 1 Concentrate Factor V activity results multiplied by dilution factor (IU/mL)

(Samples pre-diluted 1/20 using Siemens, IL or CRYOcheck factor deficient plasma)

	Innovin/Siemens	Recombiplastin/ IL	PT-Fib-HS+ / IL	Innovin/CRYOcheck
DAY 1	F V:C (IU/mL)	F V:C (IU/mL)	F V:C (IU/mL)	F V:C (IU/mL)
30 min	15.0	15.8	13.5	16.1
2 Hours	13.9	17.1	14.1	16.5
DAY 2				
30 min	14.9	17.2	14.6	15.5
2 Hours	14.4	14.7	12.2	14.7
DAY 3				
30 min	13.1	15.6	12.4	14.2
2 Hours	13.4	16.8	13.0	14.5
30 min Mean	14.3	16.2	13.5	15.3
2 Hour Mean	13.9	16.2	13.1	15.2
Overall Mean	14.1	16.2	13.3	15.3
CV (%)	5.7	6.0	7.2	6.1

Conclusions: Arrival of this therapeutic FV concentrate potentially offers a major advance for treatment of patients with FV and FV+FVIII deficiency, however our data illustrated care must be taken in selecting reagents for assay of this product.

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