Comfort-in™ needle free soft injector system	510 K application (K120687) **Sections 19:** **Performance Testing – *In Vitro* and Animal Studies**

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List of Abbreviations

BD	Becton and Dickinson Company
cm	Centimeter
°C	Degree Celcium
CI	Confidence Interval
IgG	Immunoglobulin G
LC/MS	Liquid Chromatography/Mass Spectrometry
MW	Molecular Weight
m/z	Mass to Charge Ratio
μm	Micron
mm	Millimeter
PAGE	Polyacrylamide Gel Electrophoresis
RSD	Relative Standard Deviation
SEM	Standard Error of Mean
SD	Standard Deviation
v/v	Volume per Volume

1. Structural Integrity Studies

Aim of the study:

Comfort-in™ needle-free injection system has been designed to be used with various medications. The device utilizes a spring force (mechanical pressure technology) to propel 0.5 ml (maximum volume) solution through a 0.006"(0.15 mm) orifice under a pressure of about 4150 psi, and this pressure is sufficient to inject various pharmaceutical products. However, it is also possible that this pressure may be sufficient to damage such compounds to the extent that they may be sheared, inactivated and/or lose pharmacologic activity or structural characteristics.

Therefore, the aim of this *in vitro* analytical study was to determine whether medicinal model compounds retain their physical characteristics (molecular weight and ionic charge) post-injection with the Comfort-in™ system.

Methodology of the testing:

The compounds investigated encompassed a range of molecules to make sure that a wide range of compound molecular weight was tested for a potential molecular and functional damage.

BD conventional syringe with 27 gauge needle was used as a predicate control device in this study.

The following compounds were tested with Comfort-in™ Needle-free injection system as per Table 1 below:

Table 1: Model Substances Tested

Model Substance	MW in Daltons	Source
L-Thyroxine human (T4)	776.9	Sigma T1775
Cyanocobalamin (Vitamin B12)	1,350	Sigma V6629
Human Recombinant Insulin	5,008	Sigma I0908
Myoglobin from equine skeletal muscle	17,000	Sigma M0630
Albumin from chicken egg white	44,000	Sigma A5503
Human Serum IgG	170,000	Sigma I4506

These compounds were first prepared as pristine stock solutions (1mg/ml) that had encountered neither the Comfort-in system, nor a syringe. Due to the fact that L-thyroxine was insoluble in water a 1mg/ml solution of T4 was made in weakly alkaline bicarbonate buffer (pH 9.0).

0.5 ml of samples were set up by the Comfort-in™ in accordance with the device use instructions after which the dose samples were expelled into a collecting glass vial until the needle-free syringe was completely emptied. All samples were stored overnight at 2-8°C prior to analysis.

Analysis of L-thyroxine and Vitamin B12

An electrospray LC/MS assay was developed with the following conditions (Table 2) compatible for evaluation of the molecular weights of the L-thyroxine and Vitamin B12.

Table 2: LC/MS Parameters Used for the Study

HPLC Parameters	Description
HPLC Column	Ace-5 C18, 5 µm, 2.1 mm id x 30 mm
Mobile-Phase A	0.1% formic acid in HPLC Water
Mobile-Phase B	0.1% formic acid in HPLC Methanol
Mobile-Phase Composition	Gradient from 90%v/vA:10% B to 10%A: 95%v/vB
Flow Rate	0.3 ml/min
Run Time	15 min

Mass Spectrometry Parameters	Description
Capillary Voltage	4.0 v
Cone Voltage	30 v and 60 v
Scan Range	100 amu to 1900 amu in 3 seconds

An Agilent Model 1100 high performance liquid chromatography system and Waters Quattro®-Micro triple quadrupole mass spectrometer controlled by MassLynx™ version 4.0 was used during the conduct of this study.

The following conditions were employed for analysis:

Gradient Table:

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Flow Rate (mL/min)
0	10	90	0.3
0.2	10	90	0.3
5.0	95	5	0.3
10.0	95	5	0.3
10.1	10	90	0.3
15	10	90	0.3

Mobile Phase A: 0.1% formic acid in HPLC water
Mobile Phase B: 0.1% formic acid in HPLC methanol
Column: Waters ACE-5, 5µm, 30 x 2.1 mm id
MS detection mode: ESI positive mode, scan from 100 to 1900 amu

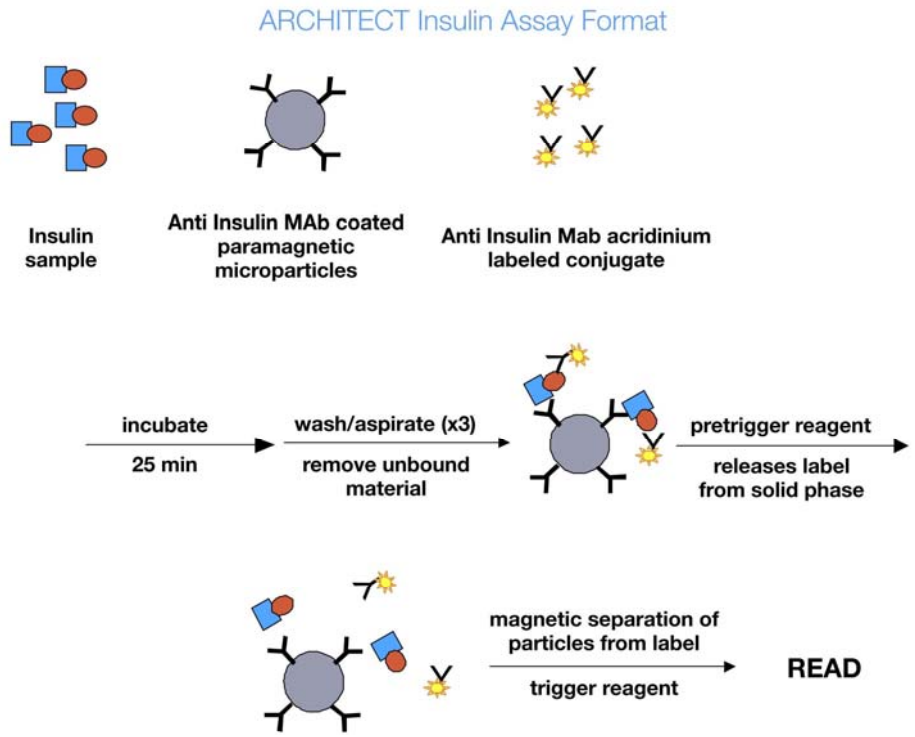
Integrations of chromatographic data and calculations of the relative retention time were performed using MassLynx™ version 4.0 and Microsoft Excel 2007.

Analysis of Myoglobin, Ovalbumin and Human Serum IgG

Pre- and post-injection samples of myoglobin, ovalbumin and human serum IgGs were analyzed by running the samples on the 12% polyacrylamide gel electrophoresis (PAGE). Broad spectrum PAGE molecular weight markers (BioRad) were run to calibrate the gel. The gel was stained with 0.25% Coomassie Blue R-250 for 4 hours followed by the de-staining for 12 hours in 5% Methanol and 7.5% acetic acid.

Immunoassay

Recombinant human insulin samples were quantitatively assayed by Abbott Architect Chemiluminescent Microparticle Immunoassay (CMIA) in accordance with the instrument manual and Architect Insulin kit insert.



The ARCHITECT Insulin assay is a one-step immunoassay to determine the presence of human insulin in the serum or plasma. Test sample, anti-insulin coated paramagnetic microparticles, and anti-insulin acridinium-labeled conjugate are combined. Insulin present in the sample binds to the anti-insulin coated microparticles and anti-insulin acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative luminescence units. A direct relationship exists between the amount of insulin in the sample and the RLUs detected by the ARCHITECT immunoassay optical system.

Results:

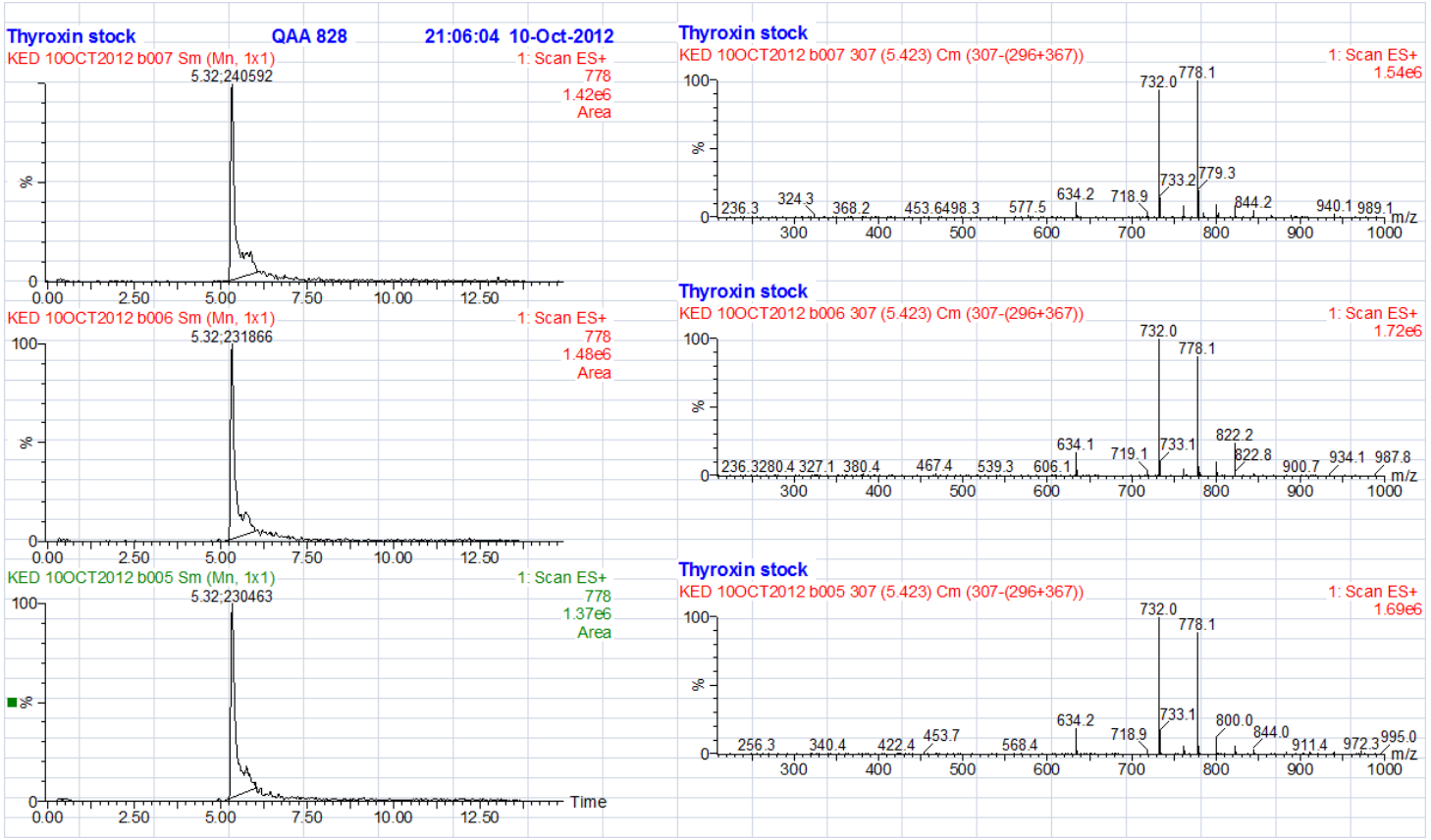
LC/MS analysis

Test Dates:	October 10-11th 2012
Test Location:	Globe Labs Inc., Vancouver BC, Canada, and BRI Biopharmaceutical, Vancouver BC Canada
Device Lot tested:	C11 20524 088
Study Director/Manager:	Dr. Gary Yalloway

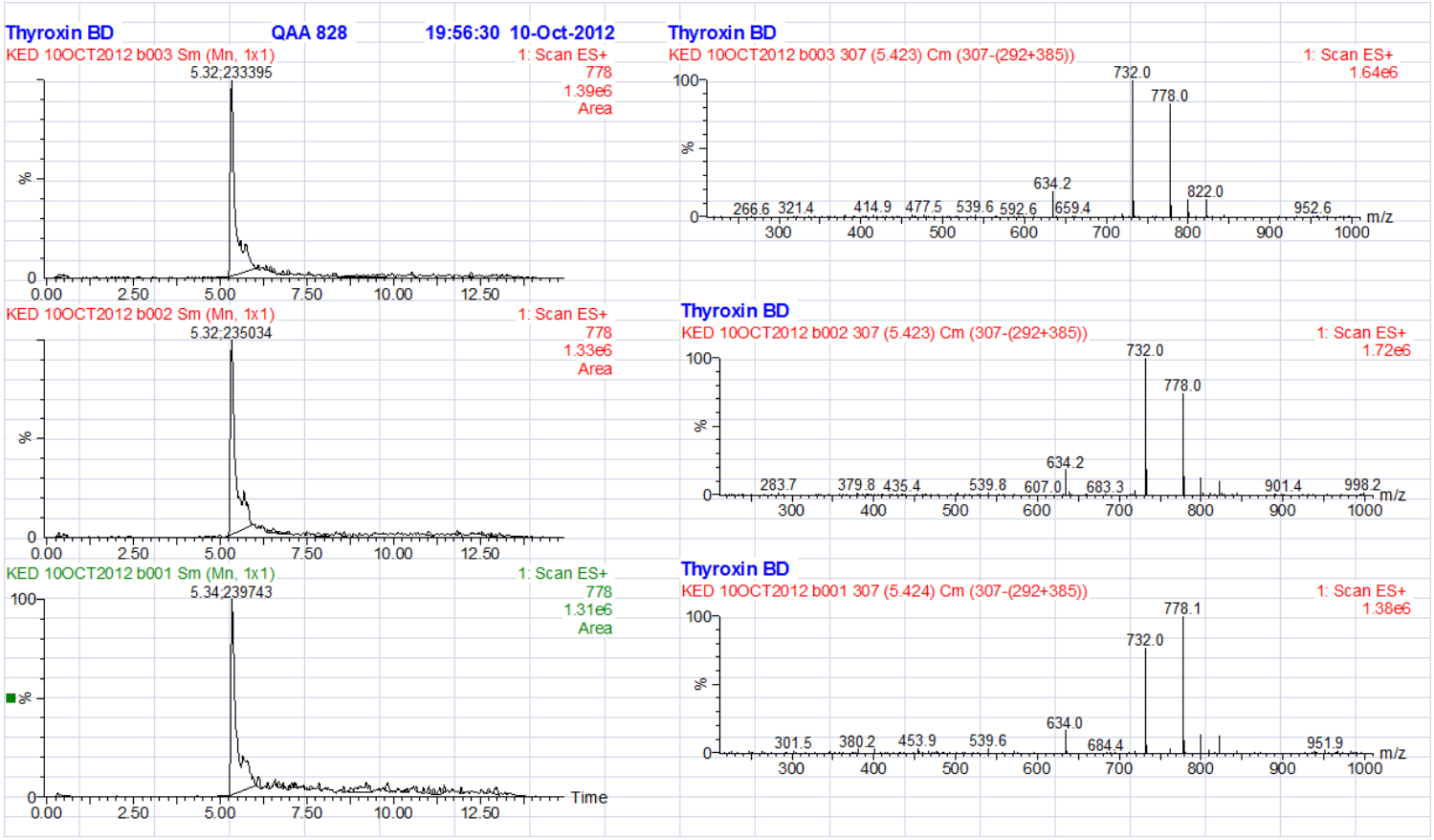
Thyroxin samples were assayed and a protonated molecular ion [M+H] observed at 778 m/z was compared and evaluated for potential differences before and after needle-free injection. Triplicate chromatograms and mass spectra of thyroxin samples labeled Stock, BD and Post-Injector are presented in the following Figure 1.

Figure 1: LC/MS Chromatograms from triplicate assay of L-Thyroxine

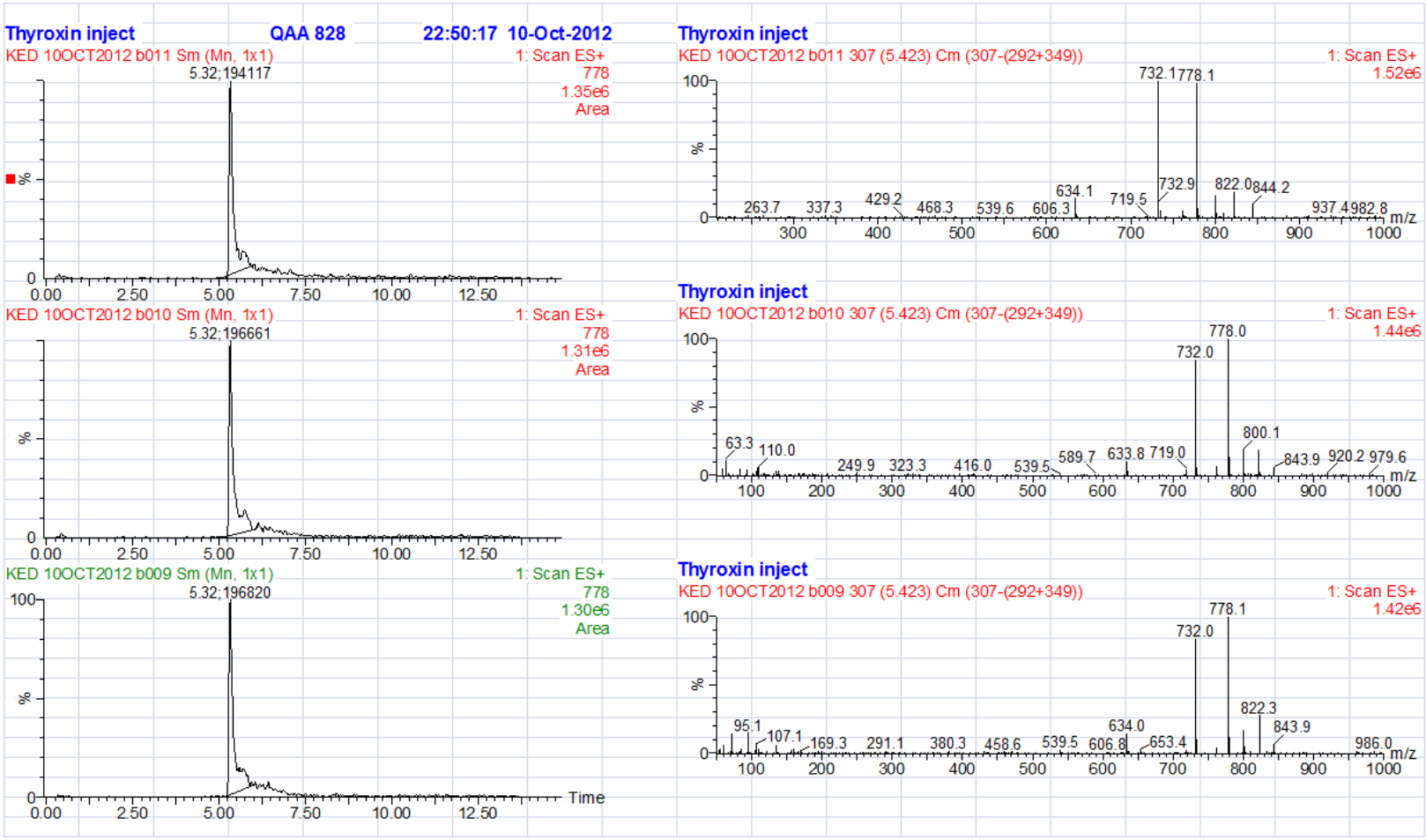
A. LC/MS chromatograms from triplicate assay showing a single predominant molecular ion [M+H] detected at 778 m/z (on the left), and the corresponding mass spectrum (right) from L-thyroxine **Stock** (pre-injection) sample.



B. Triplicate assay showing a single predominant molecular ion [M+H] detected at 778 m/z (on the left), and the corresponding mass spectrum (right) from L-thyroxine **BD sample**.



C. Triplicate assay showing a single predominant molecular ion (M+H) detected at 778 m/z (on the left), and the corresponding mass spectrum (right) from L-thyroxine **Post-Injector sample**.



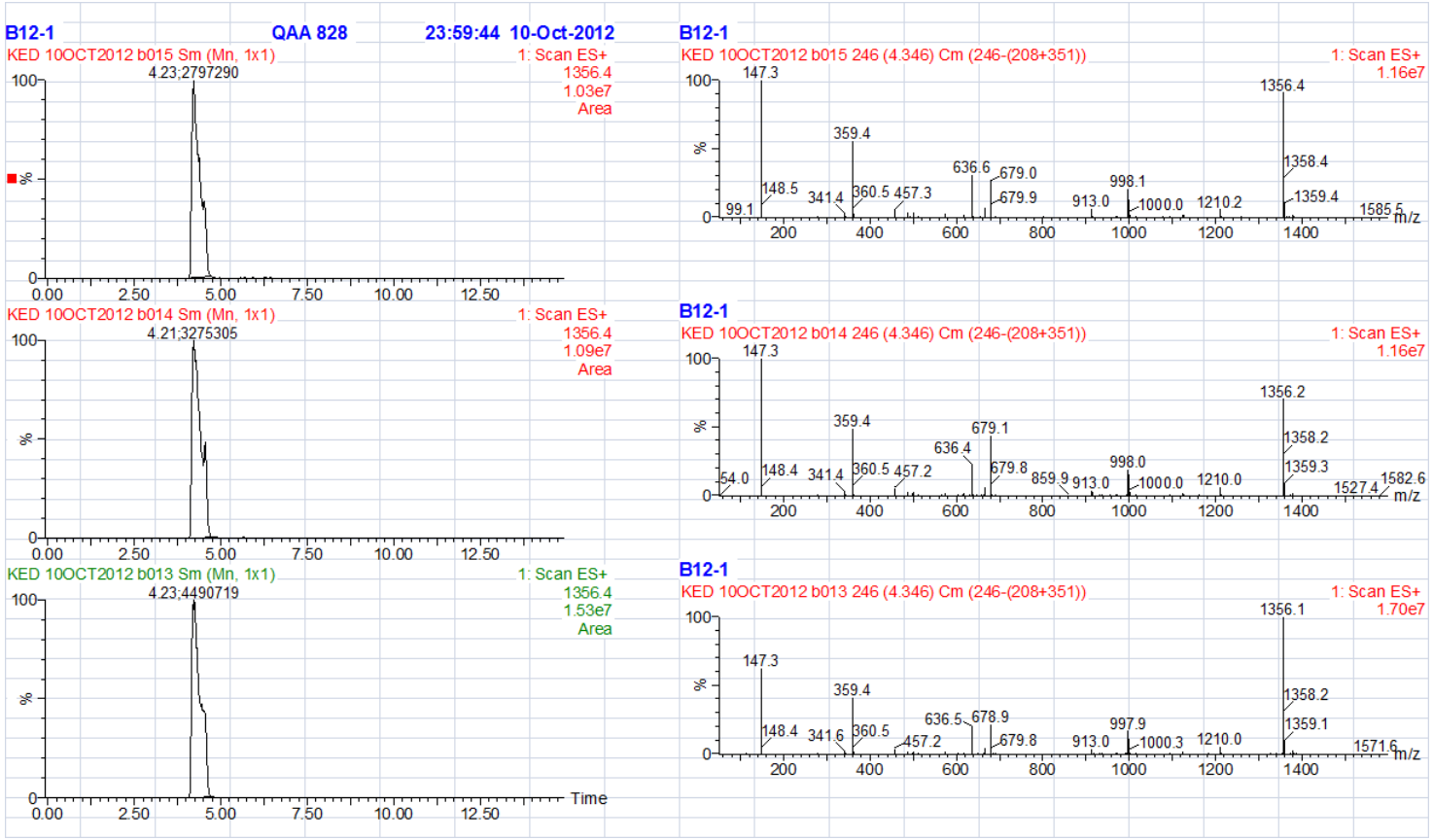
All three L-thyroxine Stock, BD Syringe and Comfort-in™ Post-Injector samples were qualitatively identical with observation of an expected protonated molecular ion [M+H] at 778 m/z.

Compound	Retention Time (in min)		
	Stock Control	BD syringe	Comfort-in™ Injector
L-Thyroxine	5.32	5.32	5.32
	5.32	5.32	5.32
	5.32	5.34	5.32
Mean	5.320	5.327	5.320
SD	0	0.0012	0

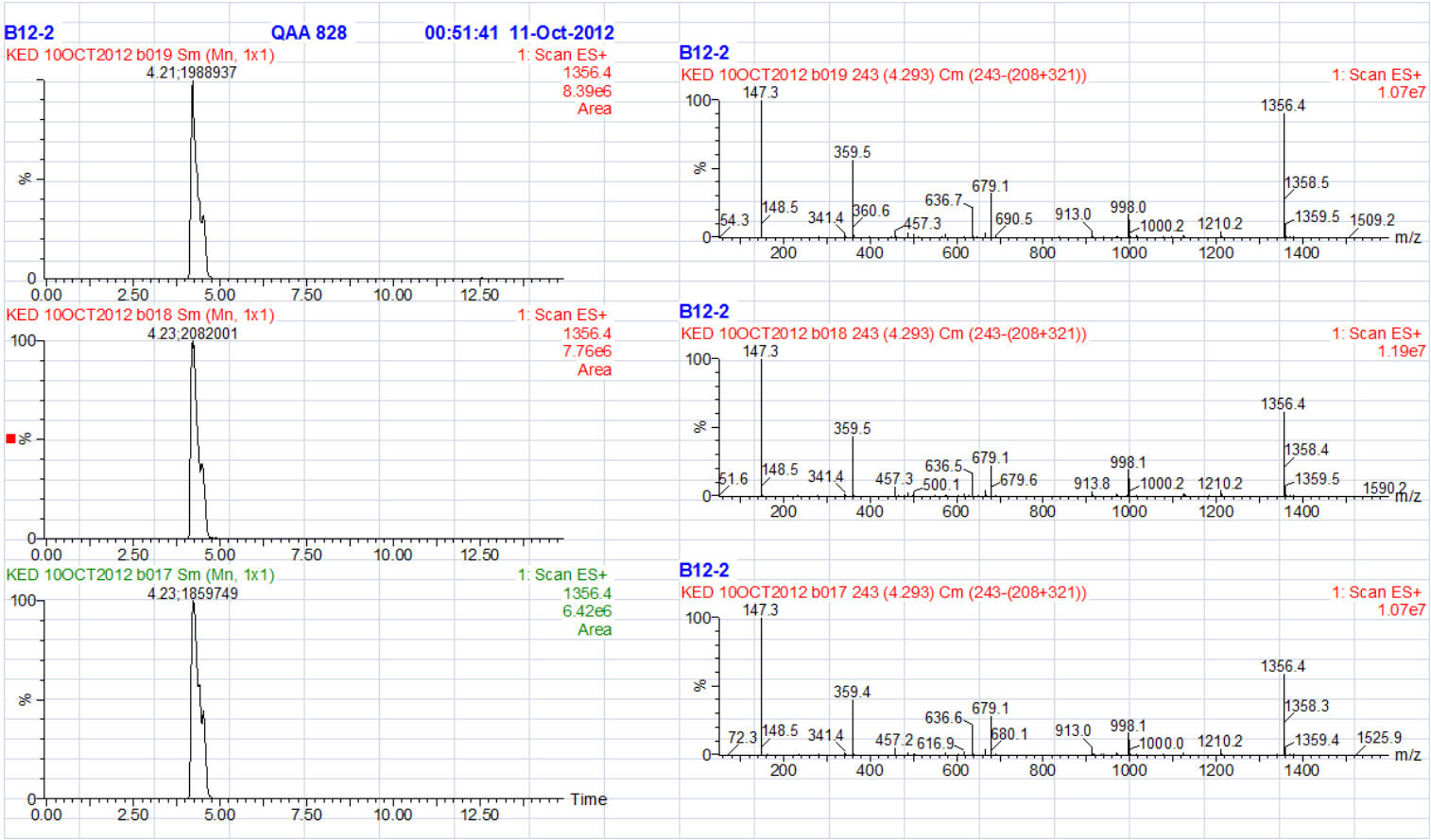
Cyanocobalamin (vitamin B12) samples were assayed and a protonated molecular ion [M+H] observed at 1356 m/z was compared and evaluated for potential differences between stock and post-BD syringe and post needle-free injector samples. Triplicate chromatograms and mass spectra of cyanocobalamin samples labeled Stock, BD and Post-Injector are presented in the Fig.2 below.

Figure 2: LC/MS Chromatograms from triplicate assay of Cyanocobalamin

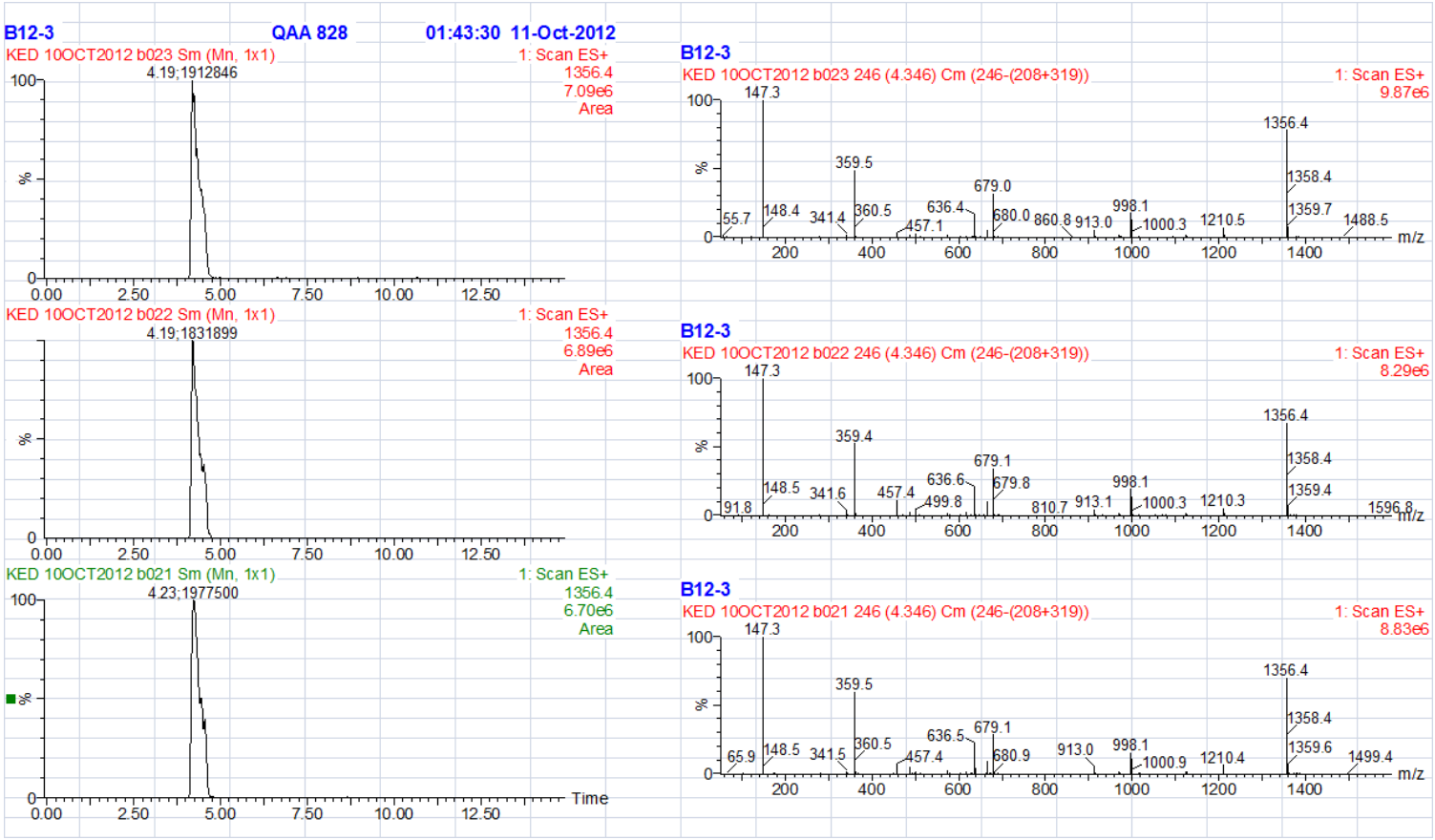
A. LC/MS chromatograms from triplicate assay showing a single predominant molecular ion [M+H] detected at 1356 m/z (on the left), and the corresponding mass spectrum (right) from vitamin B12 **Stock** (pre-injection) sample.



B. Triplicate assay showing a single predominant molecular ion [M+H] detected at 1356 m/z (on the left), and the corresponding mass spectrum (right) from vitamin B12 **BD sample**



C. Triplicate assay showing a single predominant molecular ion [M+H] detected at 1356 m/z (on the left), and the corresponding mass spectrum (right) from vitamin B12 **Post-Injector sample**



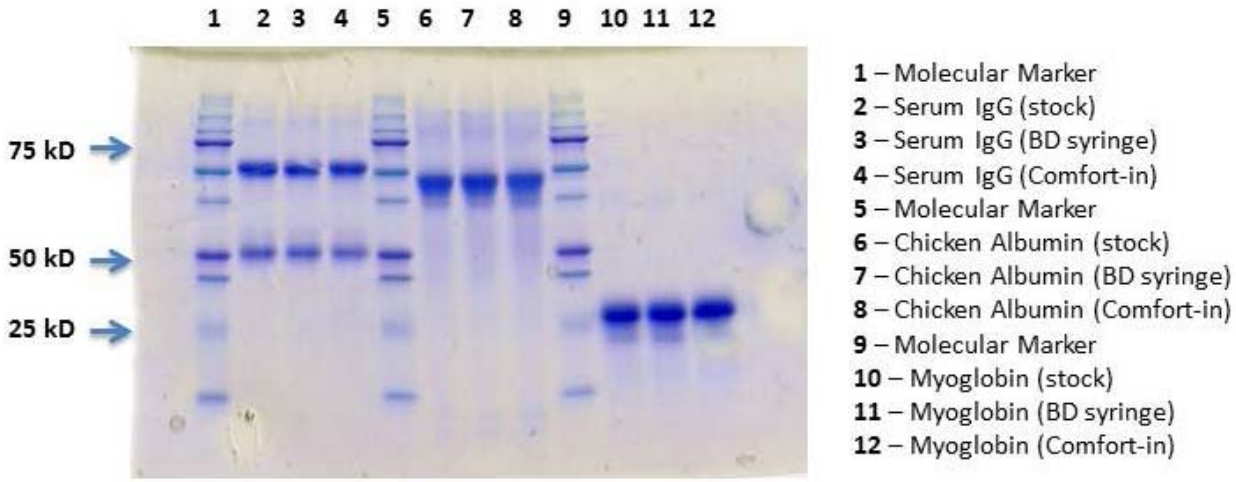
All three Cyanocobalamin (vitamin B12) Stock, BD Syringe and Comfort-in™ Post-Injector samples were qualitatively identical with observation of an expected protonated molecular ion [M+H] at 1356 m/z.

Compound	Retention Time (in min)		
	Stock Control	BD syringe	Comfort-in™ Injector
Cyanocobalamin (vitamin B12)	4.23	4.21	4.19
	4.21	4.23	4.19
	4.23	4.23	4.23
Mean	4.223	4.223	4.203
SD	0.0115	0.0115	0.0231
t-test		P=1	P=0.25

PAGE

Test Dates: September 20th 2012
Test Location: Globe Labs Inc., Vancouver BC, Canada
Device Lot tested: C11 20524 091
Study Director/Manager: Lena Wong

Figure 3: PAGE Data



Insulin Immunoassay

Test Dates: September 25th 2012
Test Location: Life Labs Medical Laboratory Services, Delta BC, Canada
Device Lot tested: C11 20524 084

Because measurement range for the Architect Insulin immunoassay was from 1 up to 300 μU/ml, all samples were diluted 1:100,000 with saline prior analysis. Obtained result numbers were corrected for the dilution factor and are presented in the Table 3 below.

Table 3: Insulin Immunoassay Data

Analyte	Insulin Concentration(in U/ml)		
	Stock Control	**BD syringe**	**Comfort-in™ Injector**
Human Recombinant Insulin	12.5	12.4	12.6
	12.6	12.6	12.5
	13.0	12.7	12.4
Mean	12.7	12.57	12.50
SD	0.265	0.152	0.100
t-test		P=0.13	P=0.22

Results Discussion:

In all analyses, L-thyroxine and vitamin B12 samples delivered by the Comfort-in™ were equivalent to the Control pre-injection stock solutions. These Control solutions were pristine stock solutions that had encountered neither the Comfort-in™, nor any syringe. Based on the LC/MS data, the use of the Comfort-in does not cause loss of molecular structure, molecular weight, or molecular charge characteristics of the tested compounds because both retention times and corresponded molecular spectra found to be similar.

The density of the protein bands (human serum IgG, myoglobin and chicken albumin) analyzed by PAGE was equivalent to both the Control and BD syringe samples. The density of the bands were visually equivalent, appeared to migrate in accordance with the assigned molecular weights as determined from the molecular markers, and no degradative products of any tested compounds were visible. Therefore, it could be concluded that molecular charge characteristics have been retained. Otherwise, any effect would have caused a shift in mobility of the compounds within the electrical field applied to the gel.

The Insulin immunoassay demonstrated that the immune-reactivity was retained suggesting that epitope moieties were maintained intact despite the high pressures of injection utilized by the Comfort-in™. If epitopes are maintained intact one can assume that overall primary and secondary structure are maintained intact. Further, protein

epitopes are generally required to be in a three dimensional conformation consistent with the original antigen used to produce and select antibodies. Since there is no loss of immune-reactivity in this study (compared to the control), the three dimensional conformation (tertiary structure) of the molecule is also maintained after injection. It is therefore plausible to conclude that structural damage and cleavage of the disulfide bonds or unwinding of the tertiary structure has not occurred as a result of injection.

2. Depth Penetration and Dispersion Study

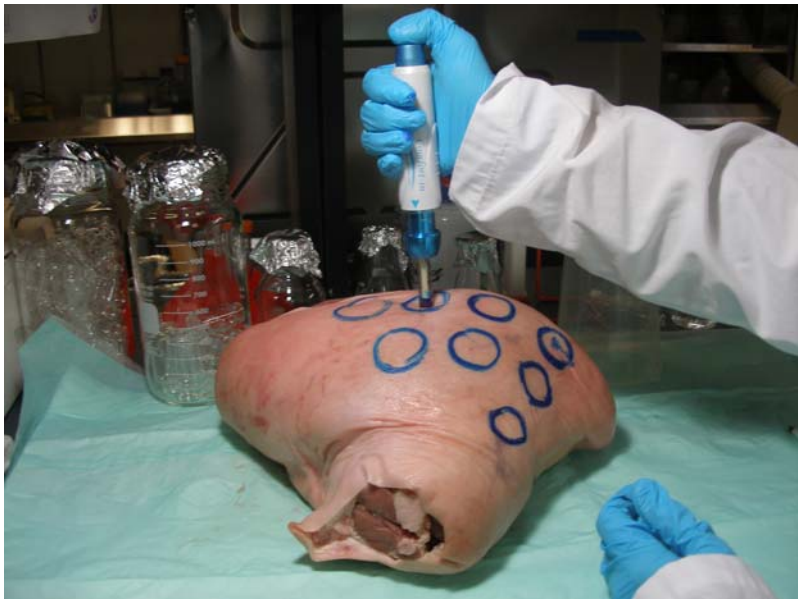
Aim of the study:

To investigate the suitability of the needle-free Comfort-in™ system, manufactured by Mika Medical Co., for subcutaneous and/or intramuscular injections of medicinal products. To this end the depth penetration of a tissue marking dye solution was determined following injection of three doses (0.1 ml, 0.3 ml and 0.5 ml) into the porcine leg. Dispersion and distribution of the dye was investigated histologically.

Methodology of the testing:

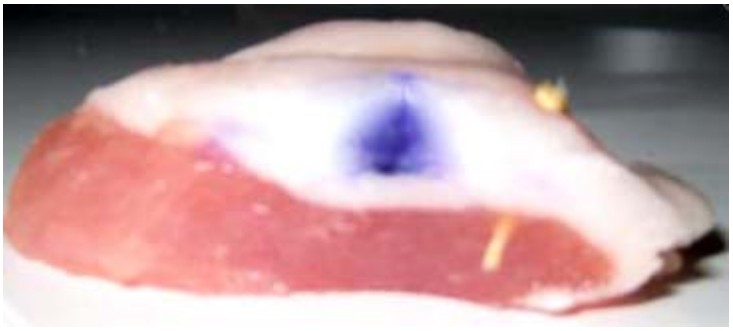
The pig legs were obtained from local farmer at Delta BC Canada. The organ was cooled during transportation to the testing laboratory. Total of four limbs were used for the experiments. Prior to the dye administration the respective areas of skin were marked with an indelible marker. The three volume of dye solution (0.1ml, 0.3 ml and 0.5 ml) was drawn into a needle-free syringe and administered with the aid of the Comfort-in™ system (Fig.4).

Figure 4: Porcine Leg used for Dye Penetration Study



The center of the injection site was quickly excised with a scalpel and depth of penetration was measured manually by a calliper ruler, as well as shape of dye dispersion was recorded (Fig.5 below)

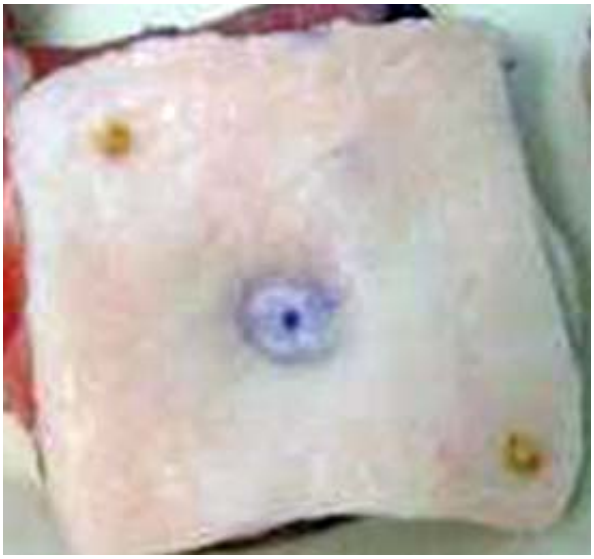
Figure 5: Cone shape of the dye penetration



Some injection sites that were not used for the depth penetration measurement (with intact center of the injection site) were cut into rectangular samples of skin approximately 25 x 25 x 25 mm in size with the attached deep musculature and fatty tissue (Fig.7).

These samples were fixed in formalin (4%) and submitted for the histological examination. The histological tissue processing and assessment was performed by the Wax-it Histology Services Inc, Vancouver BC. The histological findings were digitally photographed and commented by veterinarian pathologist.

Figure 6: Injection site sample sent to the histology examination



Results:

Date of the testing:	September 3rd 2012; Histology reported September 19th 2012
Location:	Globe Laboratories, Vancouver BC and Wax-it Histology Services Inc., Vancouver BC
Device tested:	Comfort-in™ needle-free soft injection system
Dye used:	Blue tissue marking dye (Sigma MDT-100)
Test Operator:	Michelle Su
Statistical Analysis:	MedCalc® v12.2.1

Table 4: Depth Penetration and Dispersion Testing Raw Data and Statistics

A. Depth Penetration of the porcine skin with 0.1 ml Blue Tissue Marking Dye

Blue Dye 0.1 ml	**Injector C11 20524 044**			**Injector C11 20524 045**		
Injection sites	**Depth in mm**	**Dispersion shape**	**Injection comment**	**Depth in mm**	**Dispersion shape**	**Injection comment**
Site 1	2.8	Subcutaneous Cone	dry	2.2	Subcutaneous Cone	dry
Site 2	2.9	Subcutaneous Cone	dry	3.0	Subcutaneous Cone	dry
Site 3	2.1	Subcutaneous Cone	dry	3.1	Subcutaneous Cone	dry
Site 4	2.8	Subcutaneous Cone	dry	2.8	Subcutaneous Cone	dry
Site 5	2.5	Subcutaneous Cone	dry	3.0	Subcutaneous Cone	dry
Sample size	10					
Lowest value	2.10					
Highest Value	3.10					
Mean	2.720					
95% CI	2.475 – 2.965					
Variance	0.1173					
SD	0.3425					
RSD	0.1259 (12.59%)					
SEM	0.1083					

B. Depth Penetration of the porcine skin with 0.3 ml

Blue Dye 0.3 ml	**Injector C11 20524 044**			**Injector C11 20524 045**		
Injection sites	**Depth in mm**	**Dispersion shape**	**Injection comment**	**Depth in mm**	**Dispersion shape**	**Injection comment**
Site 1	4.2	Cone-like, SC	dry	4.1	Cone-like, SC	dry
Site 2	3.9	Cone-like, SC	dry	4.4	Cone-like, SC	dry
Site 3	4.0	Cone-like, SC	dry	3.8	Cone-like, SC	dry
Site 4	4.7	Cone-like, SC	dry	4.5	Cone-like, SC	dry
Site 5	4.5	Cone-like, SC	dry	4.0	Cone-like, SC	dry
Sample size	10					
Lowest value	3.80					
Highest Value	4.70					
Mean	4.210					
95% CI	3.9955 – 4.4245					
Variance	0.08989					
SD	0.2998					
RSD	0.07121 (7.12%)					
SEM	0.09481					

C. Depth Penetration of the porcine skin with 0.5 ml

Blue Dye 0.5 ml	**Injector C11 20524 044**			**Injector C11 20524 045**		
Injection sites	**Depth in mm**	**Dispersion shape**	**Injection comment**	**Depth in mm**	**Dispersion shape**	**Injection comment**
Site 1	5.8	Cone-like, SC	dry	5.5	Cone-like, SC	dry
Site 2	5.3	Cone-like, SC	dry	6.1	Cone-like, SC	dry
Site 3	4.9	Cone-like, SC	dry	5.8	Cone-like, SC	dry
Site 4	6.0	Cone-like, SC	dry	4.9	Cone-like, SC	dry
Site 5	5.4	Cone-like, SC	dry	5.3	Cone-like, SC	dry
N	10					
Lowest value	4.90					
Highest Value	6.10					
Mean	5.50					
95% CI	5.1984 – 5.8016					
Variance	0.1778					
SD	0.4216					
RSD	0.07666 (7.67%)					
SEM	0.1333					

Figure 7: Depth Penetration with Respect to Injected Volumes

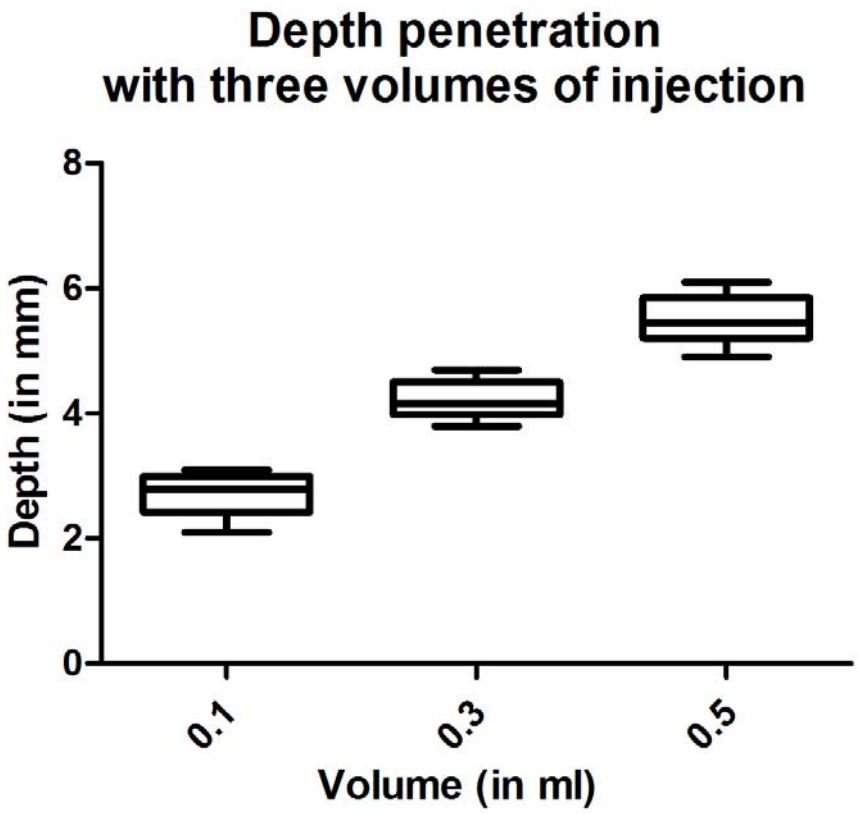
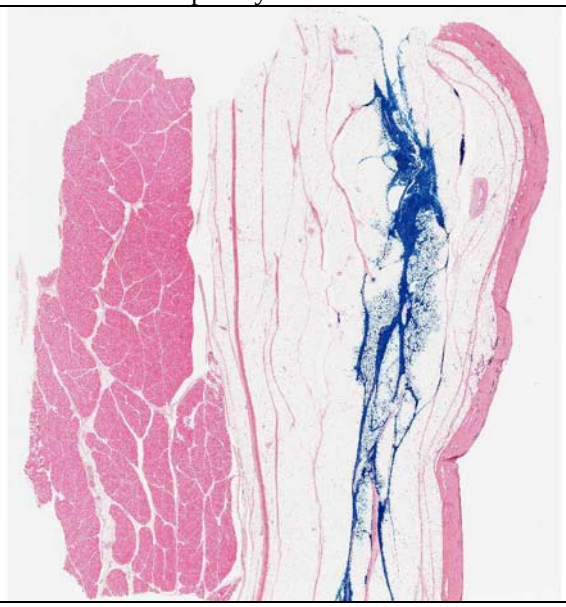


Table 5: Histological assessment

Histology	0.5 ml blue dye Injector C11 20524 044
General assessment	An excised skin sample measuring 24 x 26 x 25 mm with the attached deep musculature and adipose tissue was submitted. Lenticular sub-epidermal deposit of dye 9 mm in diameter and 5.5 mm in depth was observed. Histologically, a deposit of dye begins beneath the intact superficial epidermis. The widest portion of the dye deposit is visible in the loose adipose tissue. The deep skeletal musculature is completely free of coloration
H&E slide	

Discussion:

In this study the Comfort-in™ needle-free injection system manufactured by Mika Medical Co. was investigated. The objects of investigation were two lots of the current model of Comfort-in™ which allows delivery of at least three volumes through the 0.15 mm (0.006 inch) orifice nozzle. The main focus of the study was the determination of the depth of penetration of a dye solution following administration with Comfort-in™ system. To test this three volumes (0.1 ml, 0.3 ml and 0.5 ml) were injected into the limbs of killed slaughter pigs.

A direct dependence of the depth of penetration on the volume administered was observed. The investigation also showed that dye penetrated subcutaneously and with cone-like shape. The Comfort-in™ system was efficient and safe to handle. The range of administered volumes were reliably injected.

Histological examination further confirmed subcutaneous dispersion of the dye.