

EQUIDYNE SYSTEMS INC.
TECHNICAL REPORT TR-105
Retention of structural/potency characteristics
of Insulins

REFERENCE: ANALYTICAL INVESTIGATIONAL PROTOCOL #EquA - 003
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I. INTRODUCTION AND PURPOSE

The Equidyne Systems, Inc. **INJEX™** device is an FDA-approved (for sub-cutaneous injections) needle-free delivery device which utilizes a mechanical pressure technology to propel 0.05 –0.5ml of solution through a 0.006" orifice under a pressure of ~3000 psi. Previously, trials have been performed by various pharmaceutical companies and Equidyne to determine whether compounds will be "sheared", inactivated, or otherwise damaged due to the pressure of injection. The previous study performed by Equidyne (Protocol EquA-001) demonstrated no detectable effect on various model compounds spanning a molecular weight range of 700 to ~1 Million daltons.

Since that time, various sources have suggested that insulins are highly likely to be damaged by jet injection methodologies, especially those insulins that are zinc or isophane crystal suspensions. Such information was either anecdotal or based on experience with jet injectors other than the **INJEX™**.

Therefore, the present study was designed to determine whether multiple forms of insulin retain their molecular structure and/or potency post-injection with the **INJEX™** needle-free delivery device. Comparisons to delivery via a standard syringe as well as a control retention were performed.

II. SCOPE

The methods, observations, results, and conclusions in this report are applicable to evaluation of molecular damage to various types of rapid, short, intermediate, and long-acting insulins as a result of injection via the **INJEX™** in comparison to a control and a standard needle syringe.

III. ACRONYMS AND DEFINITIONS

- Rapid acting insulins – Insulin formulations with pharmacokinetic profiles resulting in total elimination within 8 hours (from euglycemic clamp studies in healthy individuals)
- Short acting insulins – Insulin formulations with pharmacokinetic profiles resulting in total elimination within 12 hours (euglycemic clamp studies)
- Intermediate acting insulins – Insulin formulations with pharmacokinetic profiles resulting in total elimination within 20-22 hours (euglycemic clamp studies)
- Long-acting insulins – Insulin formulations with pharmacokinetic profiles resulting in total elimination within 24-26 hours (euglycemic clamp studies)

IV. PERFORMED BY

This study was performed, managed, and analyzed by Mark J. Sarno, Scientific Consultant to Equidyne Systems and Founding Partner, Vision Biotechnology Consulting.

V. REFERENCES

Protocol EquA- 003

VI. EQUIPMENT, MATERIALS AND METHODS

STUDY SYNOPSIS

The study is designed as an in-vitro test using an immunometric method to indicate outcome.

EQUIPMENT

Equidyne **INJEX**TM 0.3 and 0.5 ml injectors, reset boxes, appropriately sized ampules, and small vial adapters were required for this study. In addition, Becton-Dickinson 0.5ml syringes and 28 gauge needles were required.

SUMMARY OF METHODS

A. MATERIALS

Insulins investigated encompassed a range of sources (porcine, bovine, human recombinant), activities (rapid acting, short acting, intermediate acting and long-acting), formulations (solutions, crystal suspensions) and manufacturers (Eli Lilly, Novo Nordisk). The nominal concentration (label claim) for all drugs is 100 Units/milliliter (U/ml). These compounds are described in more detail in **Table 1** below.

TABLE 1: Study drug descriptions

INSULIN	ACTIVITY	SOURCE	FORMULATION	MANUFACTURER
Humalog	Rapid	Human	Solution	Eli Lilly
Velosulin	Rapid	Human	Solution	Novo Nordisk
Humulin R	Short	Human	Solution	Eli Lilly
Novolin R	Short	Human	Solution	Novo Nordisk
Lente Iletin I	Intermediate	Bovine/Porcine	Crystal suspension	Eli Lilly
Humulin N	Intermediate	Human	Crystal suspension	Eli Lilly
Humulin L	Intermediate	Human	Crystal suspension	Eli Lilly
NPH Iletin I	Intermediate	Bovine/Porcine	Crystal suspension	Eli Lilly
Humulin U	Long	Human	Crystal suspension	Eli Lilly

B. MEASUREMENT METHOD

Insulins were measured by radioimmunoassay (RIA). The assay utilizes guinea pig antiserum specific for insulin as the primary antibody and radioiodinated recombinant human insulin as tracer. A second antibody specific for guinea pig immunoglobulin is used to precipitate immune complexes. Each sample was tested in duplicates. Testing was performed by the Esoterix unit of Endocrine Sciences (Calabasas, CA). All samples required dilutions in order to achieve concentrations within the quantitative linear range of the assay (0-60 μ U/ml).

C. TEST INJECTIONS

For each compound and each delivery method, 6 deliveries were performed, i.e. 0.3 ml was delivered 3 times into two Type I glass receptacles by a Becton-Dickinson 0.5ml syringe fitted with a 28 gauge needle, another 6 aliquots of 0.3ml were delivered into two Type I glass receptacles by the **INJEX™** 0.3ml injector, and another 6 aliquots of 0.5ml were delivered into two Type I glass receptacles by the **INJEX™** 0.5 ml injector. A pre-delivery retention of each compound was kept as a control. The **INJEX™** and syringe test aliquots were then compared to the retention control using the immunoassay method described above.

D. STATISTICAL METHODS

Standard statistical methods were employed to compare the results (U/ml) from the **INJEX™** (0.3 and 0.5ml models), syringe, and control solutions. Means, standard deviations, and percent coefficients of variation (%CV) are reported.

Additionally, insulin values from the **INJEX™** (0.3 and 0.5ml models), syringe, and control were compared parametrically using the Students-t test. Differences between delivery methods and vs. the control are reported. $P < 0.05$ was considered significant.

VII. RESULTS

Table 2 displays the results from the immunoassay analysis. Means and standard deviations (SD) are reported for each insulin injected with the **INJEX™** devices (0.3 and 0.5ml models), the syringe, and the control retention.

TABLE 2: Immunoassay results by insulin and injection device, or control

INSULIN	Control Mean U/ml (SD)	0.3ml INJEX™ Mean U/ml (SD)	0.5ml INJEX™ Mean U/ml (SD)	Syringe Mean U/ml (SD)
Humalog	89,94 (4,64)	86,54 (9,35)	88,39 (7,79)	87,94 (5,77)
Velosulin	88,98 (4,61)	98,94 (6,30)*	100,45 (2,41)*	90,47 (2,71)
Humulin R	93,33 (2,36)	94,53 (4,84)	102,70 (2,50)*	89,79 (1,81)
Novolin R	91,42 (0,28)	99,20 (4,86)	92,36 (10,25)	92,79 (0,76)
Lente Iletin I	96,16 (3,62)	86,34 (13,51)	83,60 (3,31)	92,72 (9,61)
Humulin N	104,86 (0,11)	92,57 (11,34)	89,87 (4,83)	93,88 (10,58)
Humulin L	104,77 (3,01)	97,66 (14,57)	99,67 (10,00)	101,08 (10,85)
NPH Iletin I	87,19 (0,46)	88,29 (13,86)	89,45 (6,89)	78,21 (5,0)
Humulin U	110,33 (1,89)	109,52 (0,88)	101,01 (10,19)	103,35 (6,51)
* $p < 0.05$ vs. control				

The results demonstrate no loss in insulin potency after injection by either the **INJEX™** devices or the syringe in comparison to the control. In fact, the only statistically significant differences found (Students-t $p < 0.05$) were significantly higher insulin values for Velosulin and Humulin R solutions injected via **INJEX™** devices vs. the control.

Figures 1-4 display the results graphically by insulin activity (rapid, short, intermediate, or long acting; errors bars = 1SD)

FIGURE 1: Values (U/ml) post-injection of rapid acting insulins by device

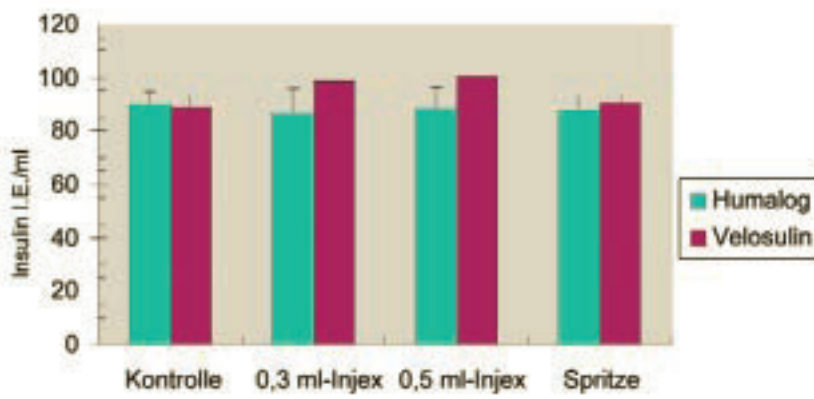


FIGURE 2: Values (U/ml) post-injection of short-acting insulins by device

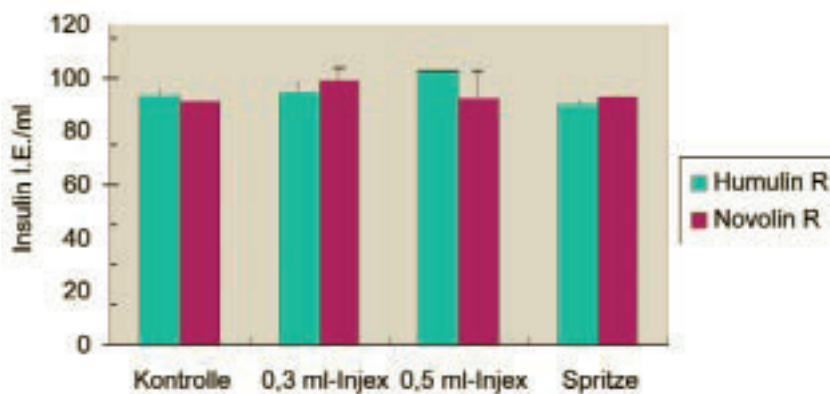


FIGURE 3: Values (U/ml) post-injection of intermediate-acting insulins by device

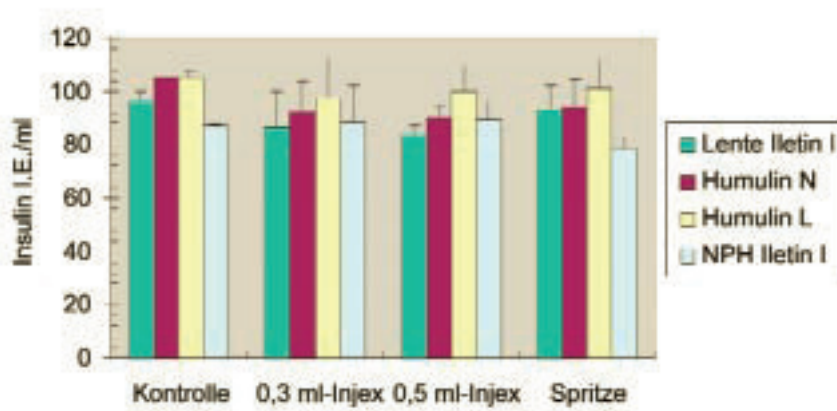
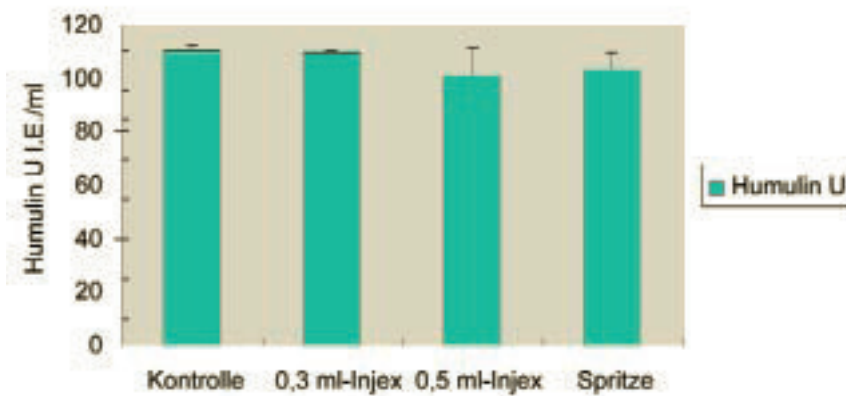


FIGURE 4: Values (U/ml) post-injection of long-acting Humulin U by device



VIII. CONCLUSIONS AND RECOMMENDATIONS

This in-vitro study demonstrates complete retention of molecular identity of insulin molecules injected via the **INJEX™**. Multiple types of insulins were explored including insulins from varying sources, of varying activities, of varying formulations, and produced by various manufacturers. In each case, the immunoreactivity was retained, thus suggesting that epitope moieties were maintained intact despite the high pressures of injection utilized by the **INJEX™** methodology.

It is a logical extension that if epitopes are maintained intact, that the overall primary and secondary structure of the molecule is likely intact. To explain further, molecular epitopes for peptide/protein molecules are generally on the order of tens of amino acids. Since insulin is a dipeptide on the order of 50 amino acids, it is unlikely that any shearing can have occurred.

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 immunoreactivity in this study, the three dimensional conformation (tertiary structure) of the molecule is also maintained after injection. This is a profound finding since insulin is a complex dipeptide protein with two disulfide bridges linking the polypeptide chains. It is therefore logical to conclude that structural damage (cleavage of the disulfide bonds or unwinding of the tertiary structure) has not occurred as a result of injection.

However, to further test the ability of the **INJEX™** to safely and efficaciously deliver Insulin, human trials are indicated. These should likely be performed as euglycemic clamp studies in healthy adult individuals. Pharmacokinetics should be measured for insulins delivered both via the **INJEX™** and standard needle syringe.

Respectfully submitted,

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