# *PharmOptima LLC*

Value-Driven Research Services

SMA FOUNDATION

# Measurement of Survival Motor Neuron in Whole Blood

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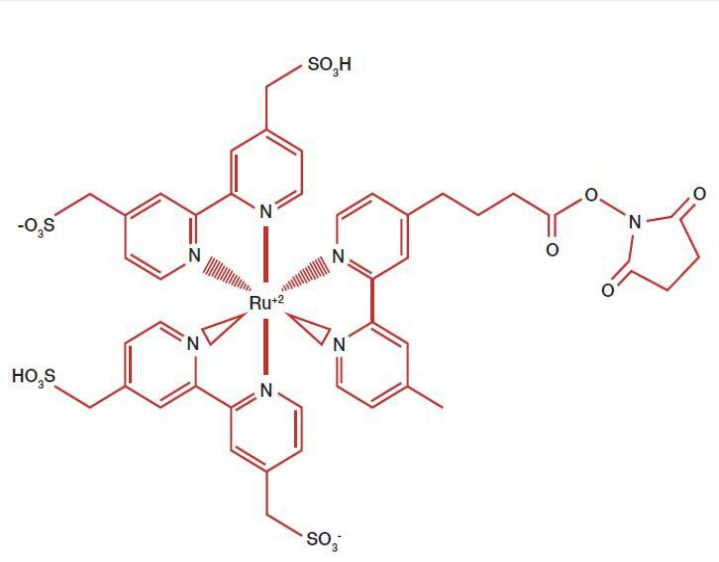
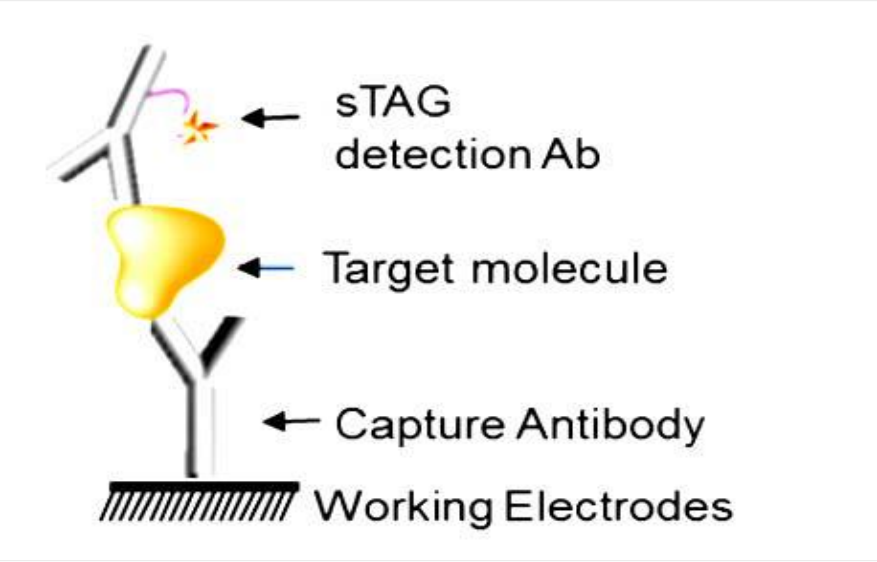
# Meso Scale Discovery Platform Electrochemiluminescence (ECL)

Signal: Caged Ruthenium tag emits light in response to an electric current

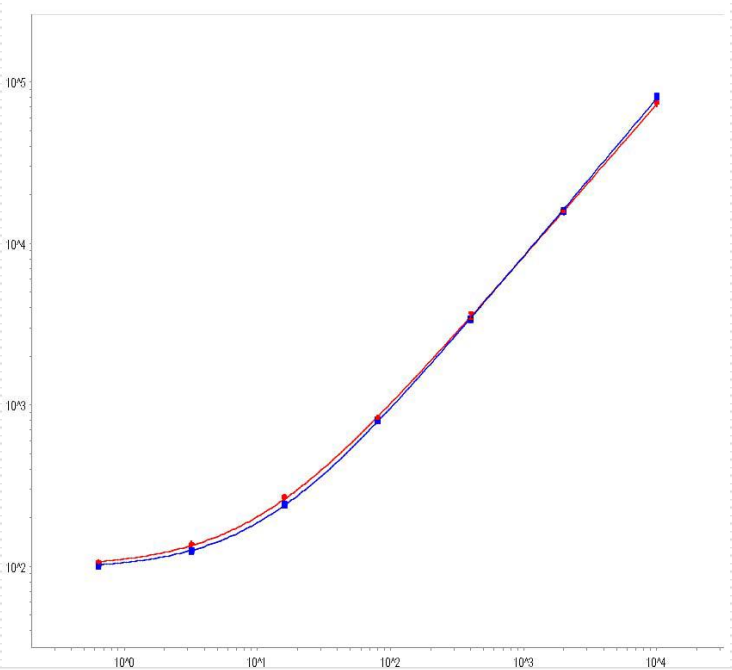
Assay Format: Sandwich Immunoassay



# SMN-ECL Assay Characteristics

**Comparison of replicate SMN Standard curves**




Capture antibody: mouse monoclonal (2B1) anti-human SMN

Detection antibody: rabbit polyclonal anti-human SMN

Sensitivity: lower limits of detection 2-3 pg/ml

Dynamic range: 3-10,000 pg/ml

Assay time: 3-4 h

Format: 96 well plate

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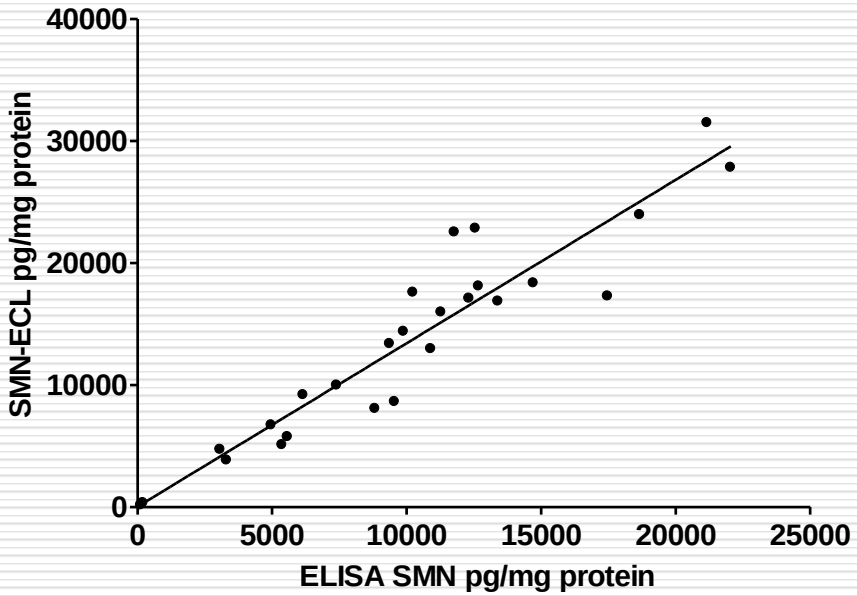
# SMN-ECL Assay Correlates With SMN ELISA

**SMN-ECL compared to SMN-ELISA in human PBMC lysates**




SMAF/PharmOptima Study Report 2011-096: Collection, processing, and ELISA analysis of SMN from PBMC, peripheral blood mononuclear cells, from SMA, spinal muscular atrophy, patients enrolled in a pilot study at Jasper Clinic

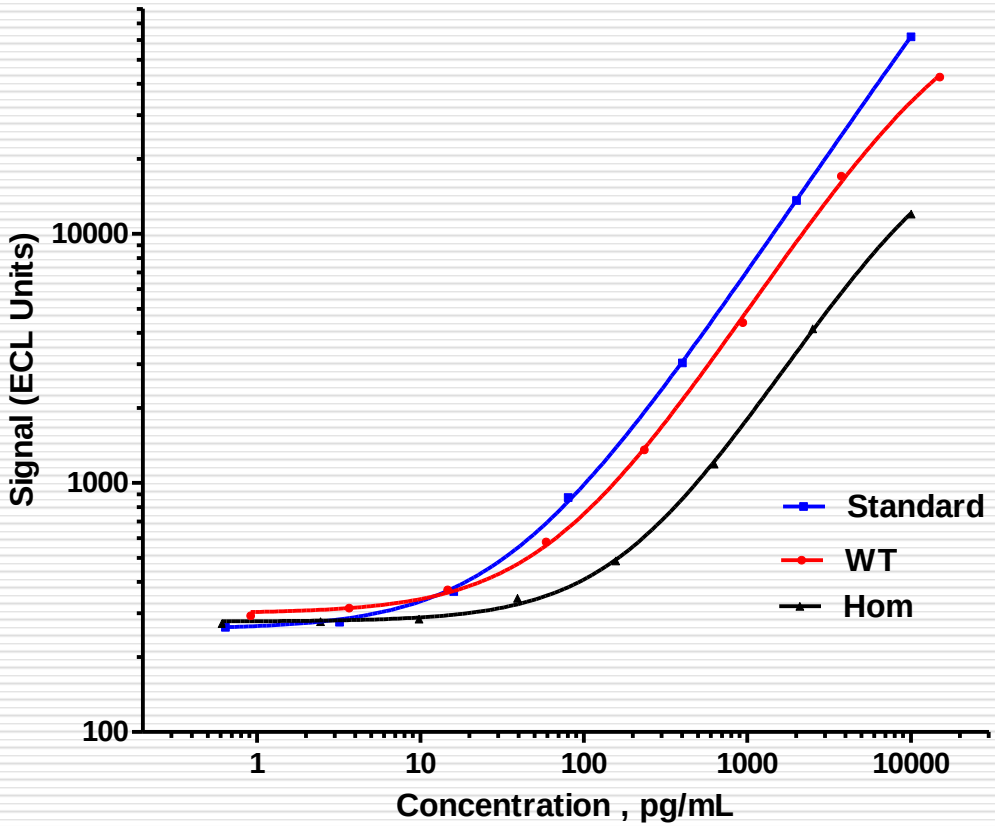
# Effect of whole blood contamination on SMN levels in C/C mouse plasma

| Sample | Visual Appearance | Mean [SMN] pg/ml | Standard Deviation |
|---|---|---|---|
| Plasma only | amber | 14.5 | 7.1 |
| Plasma, 2% WB lysate spike | pink | 154.3 | 11.4 |
| Plasma, 4% WB lysate spike | pink | 412.5 | 18.3 |

# Effect of whole blood contamination on SMN levels in C/C mouse cerebral spinal fluid (CSF)

| Animal ID | Visual Appearance | Mean [SMN] pg/mL | Standard Deviation |
|---|---|---|---|
| HOM-A18 | pink | 330.7 | 5.3 |
| HOM-A26 | clear colorless | 69.1 | 7.9 |
| HOM-A48 | clear colorless | 62.9 | 0.0 |

# Parallelism between a SMN standard curve and a mouse whole blood dilution curve




| SMN spike (pg/ml) final concentration | % spike recovery |
|---|---|
| 500 | 90.7 |
| 250 | 83.7 |
| 100 | 90.8 |

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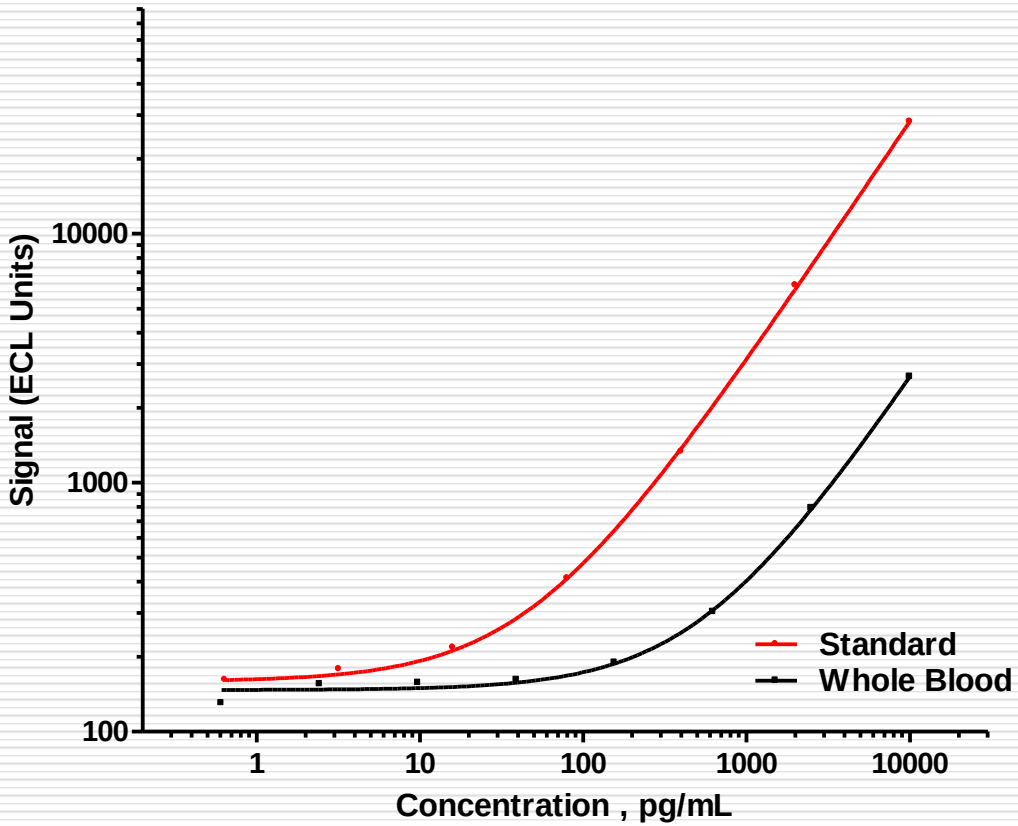
# Parallelism between a SMN standard curve and a human whole blood dilution curve




| SMN spike (pg/ml) final concentration | % spike recovery |
|---|---|
| 5000 | 95.9 |
| 1000 | 97.2 |
| 200 | 102.5 |

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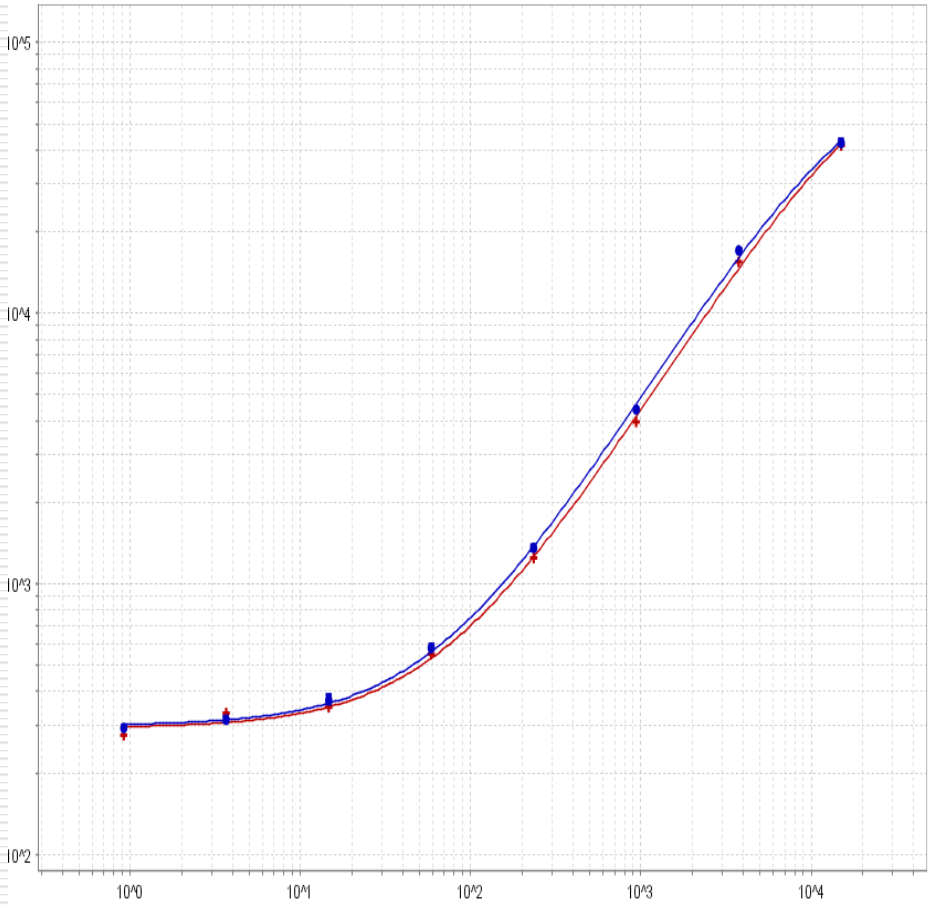
# Comparison of mouse whole blood dilution curves following a single freeze/thaw




- **No Freeze Thaw**
- **Freeze Thaw**

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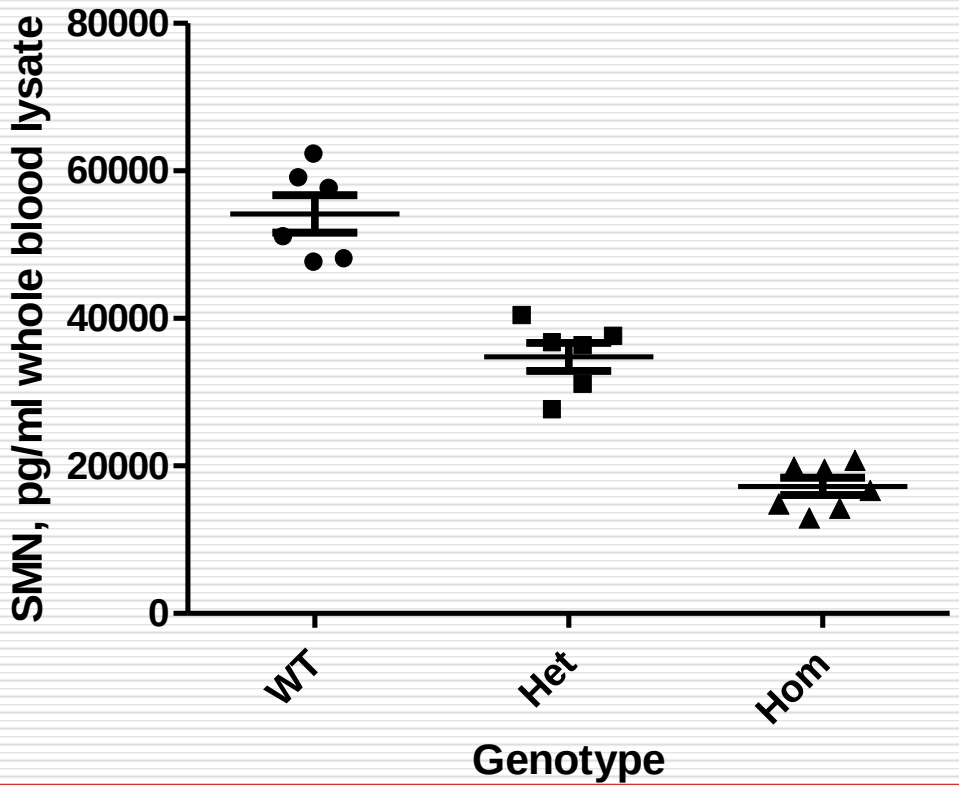
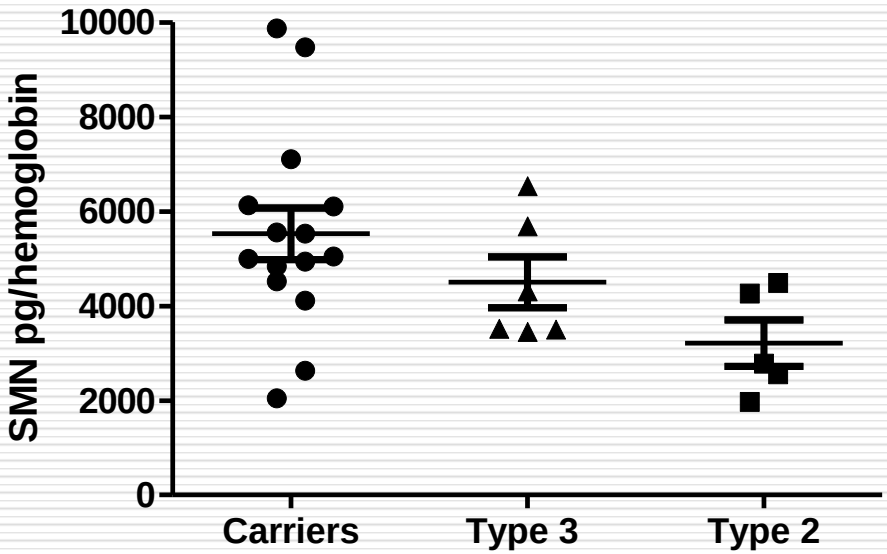
# SMN in mouse whole blood – SMN changes in C/C mouse genotypes

- Comparison of SMN levels in wild type, heterozygous and homozygous C/C mice results in statistically significant differences between the genotypes



# SMN in human whole blood
# SMN changes in human carriers & patients

- Frozen human whole blood from SMA carriers, Type 2 and Type 3 SMA patients demonstrated statistically significant differences between carriers and Type 2 patients
- This difference was not seen using PBMCs from the same samples




References


- SMAF/PharmOptima Study Report 2011-096: Collection, processing, and ELISA analysis of SMN from peripheral blood mononuclear cells (PBMC) from SMA (spinal muscular atrophy) patients enrolled in a pilot study at Jasper Clinic
- Evaluation of Peripheral Blood Mononuclear Cell Processing and Analysis for Survival Motor Neuron Protein (Kobayashi et al 2012) PLOS ONE November 2012 | Volume 7 | Issue 11 | e50763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# Whole blood fractionation study

Methods:

- Platelet isolation: Low speed centrifugation and harvest of platelet rich plasma
- PBMC isolation:
  - Ficol gradient centrifugation
  - Buffy coat harvest from low speed centrifugation
- Reticulocyte isolation: Anti-CD71 coated magnetic bead (Myltenii)
- Granulocyte isolation: Treatment of red blood cell pellet from low speed centrifugation with erythrocyte lysis buffer.
- RBC isolation:
  - Ficol interference with the SMN-ECL assay.
  - RBC fraction contribution to total SMN signal determined by subtraction

# Blood fractionation by centrifugation (Preliminary Results)

| Cell Type | Number of cells per mL | Circulation Lifetime | % Total Whole Blood SMN per mL |
|---|---|---|---|
| RBC | $4 – 6 \times 10^9$ | 100 – 120 days | 20 – 40 |
| Granulocyte | $2 – 8 \times 10^6$ | hours – days | |
| Reticulocyte | $4 – 6 \times 10^7$ | 1 day | 2 |
| Platelet* | $1.5 – 4 \times 10^8$ | 5 – 9 days | 40 |
| PBMC | $0.2 – 3 \times 10^6$ | Varies greatly | 20 |

*Denis et al 2005 Cell vol 122, 379-391: Escaping the nuclear confines: Signal-dependent pre-mRNA splicing in anucleate platelets.

# Summary

- SMN can be measured in whole blood
- As little as 5µl whole blood sufficient for assay
- Low sample requirements allows for longitudinal studies in mice
- Statistically significant differences noted between genotypes of SMA C/C mice
- Statistically significant differences noted between Type 2 patients and carriers
- SMN appears to be distributed throughout all whole blood cell types examined

# Thank You Contributors


Kathleen McCarthy
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# Why PharmOptima?

- Project team of scientists find optimal experimental approaches
- Experts who understand the data required to move IP up the value chain
- Consultation on best approaches to solve difficult challenges
- Scientists with broad expertise to execute your studies
- Years of experience developing, validating and executing GLP studies
- Full attention of a small company allowing rapid study start and completion and competitive pricing

# Contact Us


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