CardioGenics Inc.

Novel paramagnetic beads…..
For optimal light collection

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Background: Market

Due to simplicity of use, paramagnetic particles (PMP) are employed in:

- *The In-Vitro-Diagnostics testing market*

  Immunoassays/DNA testing in clinical laboratory for almost all disease segments

- *Life sciences research market*

  Cell & nucleic acid separation

  Protein purification

  Sample enrichment

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Background: Biomedical Market

- Global IVD market estimated at $42.9 billion in 2007, expected growth to $56 billion by 2012

  (all laboratory products, POC, OTC devices)
- Immunoassay testing market is 28% of the IVD testing market
- Immunoassay analyzers that employ both paramagnetic particles (PMP) and light reactions represent more than 80% of this market
- Estimated PMP market of about $1 billion (clinical use only)

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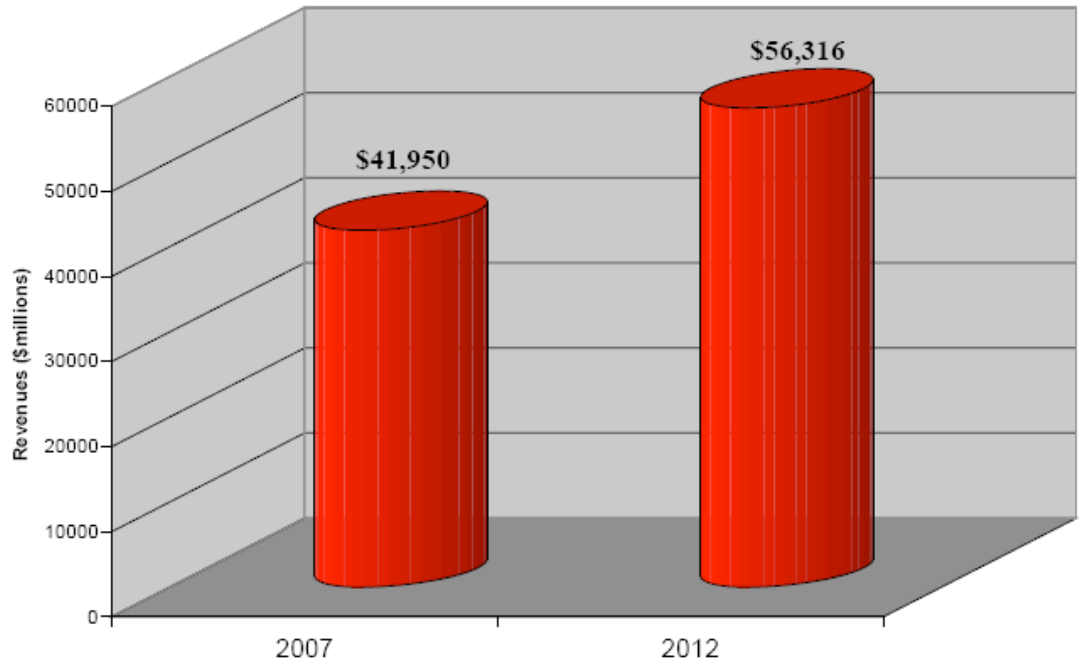
The In-Vitro-Diagnostics Market

Figure 1-1

World In Vitro Diagnostics Market 2007-2012



Source: The Kalorama Report, 2008

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Background: Biomedical Utilization

Paramagnetic particles are:

- A significant reagent for the IVD industry
- Used as a solid phase to link biological agents (testing/separation)
- Used in 90% of clinical testing machinery (immunoassay/DNA)
- Used in 90% of laboratory analyzers that use light measurements
- Introduced to the biomedical market about 2 decades ago

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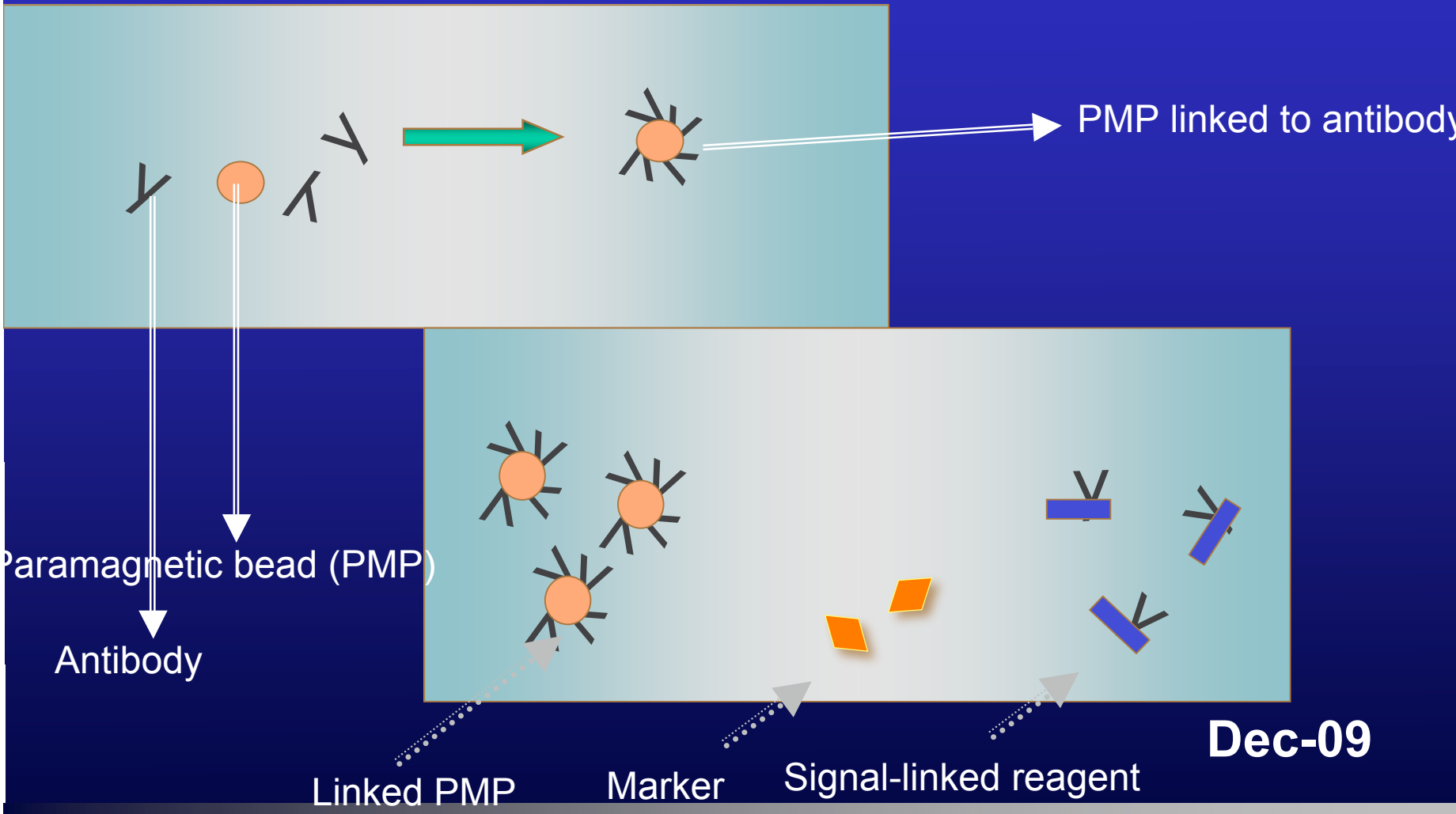
Novel Paramagnetic Beads



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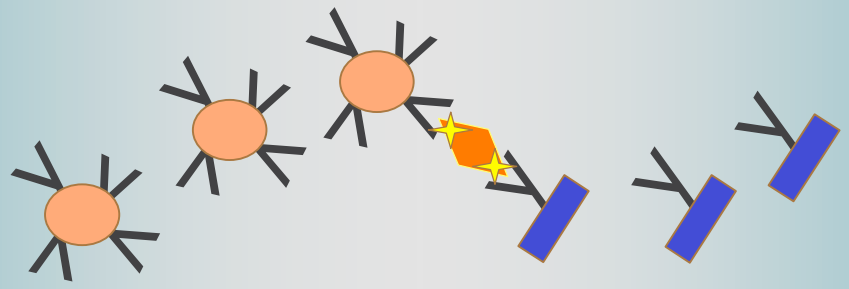

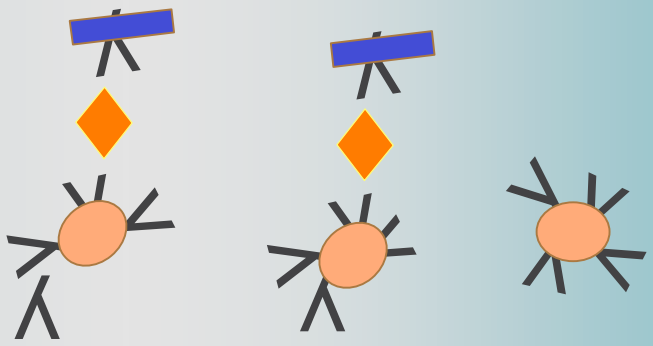

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Product: White paramagnetic beads

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Product: White Paramagnetic Beads

- Color conversion (black ⟹ white) of paramagnetic bead
- Beads plated with silver, then polymer encapsulation
- For QL care use, a multilayer polymer coating for stability
- Manufactured by a simplified process
- Beads of various sizes (1-50 micron)
- Scaled up batch-sizes to commercial lots
- 24-month developmental process, supported by NRC through IRAP grants (4 in total)

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Product: White Paramagnetic Beads

Color conversion: silver plating

- A proprietary electroless silver plating process
- Controlled silver deposition, controlled reflectivity
- No effects of deposited silver on magnetic properties
- Chemical/mechanical stability optimized
- Silver layer protection (passivation)

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Product: White Paramagnetic Beads

Internal polymer coating (encapsulation) process

- Proprietary multilayered polymer coating process
- Minimum of three polymer layers to control:
  - layer thinness/brightness
  - functional group density
  - ion leakage/ion capture
- A combination of electrostatic, covalent bonding/cross-linking coating procedure - enhance bonding and control layer thickness
- A hydrophilic polymer surface - minimizes non-specific binding
- Tailored surface functionality - different functional groups for various linking chemistries

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Product: White Paramagnetic Beads

Characterization

- Silver layer coating has no effect on magnetic moment
- 80% magnetic material - quick magnetic response
- No deterioration/peeling/loss of silver/polymer in salt high solutions
- Coated layers are stable in buffers >12 months
- No ion leakage (assessed by enzyme)
- Tailored functional surface coating (Amino, Carboxyl):
  - to influence colloidal status
  - to influence background

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Commercial Products

- Several companies commercialize paramagnetic particles
- Manufacturing processes, by encapsulation or paramagnetic pigment insertion in a latex particle
- All commercial products contain dark iron oxide material
- Three main players, several small ones
- Prices range from $900-$1500/gram of solids

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Product: White Paramagnetic Beads

Competitive Advantages

- All commercial paramagnetic particles are dark
- Maximize light collection by white color conversion
- ~ 80% loss of generated light - low testing sensitivity
- High magnetic moment, easy manipulation
- Hydrophilic surface - optimal specific and low background
- Chemically robust surface chemistry - long term stability
- Simplified manufacturing process - less costly

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Product: White Paramagnetic Beads

Compared with beads of all major suppliers, our white particles show consistent ~7-fold improved light signal to address needs of IVD industry for more sensitive tests.

Posters 41 and 42, Oakridge, AACC, 2007

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Novel Paramagnetic Beads

	Commercial products	CGI beads
Test sensitivity	Limit test sensitivity	Allow maximum light collection
Surface chemistry	Mostly hydrophilic	Hydrophilic
Manufacture	Expensive processes	Much less expensive
Magnetic moment	Very limited, up to 40% iron, cumbersome	Very high, up to 80%, easy manipulation
Ion leakage	Well documented	No ion leakage for 12 months in solution

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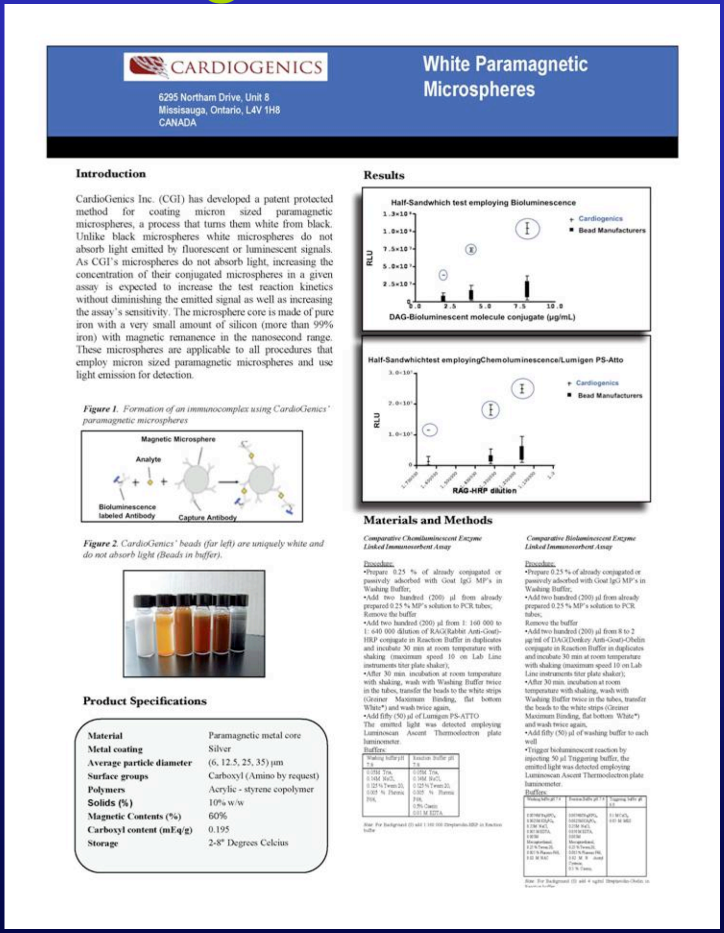
Product: White Paramagnetic Beads

Competitive Advantages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6295 Northam Drive, Unit 8
Missisauga, Ontario, L4V 1H8
CANADA

White Paramagnetic Microspheres

Introduction

CardioGenics Inc. (CGI) has developed a patent protected method for coating micron sized paramagnetic microspheres, a process that turns them white from black. Unlike black microspheres white microspheres do not absorb light emitted by fluorescent or luminescent signals. As CGI's microspheres do not absorb light, increasing the concentration of their conjugated microspheres in a given assay is expected to increase the test reaction kinetics without diminishing the emitted signal as well as increasing the assay's sensitivity. The microsphere core is made of pure iron with a very small amount of silicon (more than 99% iron) with magnetic remanence in the nanosecond range. These microspheres are applicable to all procedures that employ micron sized paramagnetic microspheres and use light emission for detection.

Figure 1. Formation of an immunocomplex using CardioGenics' paramagnetic microspheres

Figure 2. CardioGenics' beads (far left) are uniquely white and do not absorb light (Beads in buffer).

Product Specifications

Material	Paramagnetic metal core
Metal coating	Silver
Average particle diameter	(6, 12.5, 25, 35) µm
Surface groups	Carboxyl (Amino by request)
Polymers	Acrylic - styrene copolymer
Solids (%)	10% w/w
Magnetic Contents (%)	60%
Carboxyl content (mEq/g)	0.195
Storage	2-8° Degrees Celcius

Results

Materials and Methods

Comparative Chemiluminescent Enzyme Linked Immunosorbent Assay

Procedure:
•Prepare 0.25 % of already conjugated or passively adsorbed with Goat IgG MP's in Washing Buffer;
•Add two hundred (200) µl from already prepared 0.25 % MP's solution to PCR tubes; Remove the buffer
•Add two hundred (200) µl from 1: 160 000 to 1: 640 000 dilution of RAG(Rabbit Anti-Goat)-HRP conjugate in Reaction Buffer in duplicates and incubate 30 min at room temperature with shaking (maximum speed 10 on Lab Line instruments titer plate shaker);
•After 30 min. incubation at room temperature with shaking, wash with Washing Buffer twice in the tubes, transfer the beads to the white strips (Greiner Maximum Binding, flat bottom White*) and wash twice again,
•Add fifty (50) µl of Lumigen PS-ATTO
The emitted light was detected employing Luminoscan Ascent Thermoelectron plate luminometer.
Buffers:

Washing buffer pH 7.8	Reaction Buffer pH 7.8
0.05M Tris, 0.14M NaCl, 0.125 % Tween 20, 0.005 % Pluronic F68,	0.05M Tris, 0.14M NaCl, 0.125 % Tween 20, 0.005 % Pluronic F68, 0.5% Casein 0.01 M EDTA

Comparative Bioluminescent Enzyme Linked Immunosorbent Assay

Procedure:
•Prepare 0.25 % of already conjugated or passively adsorbed with Goat IgG MP's in Washing Buffer;
•Add two hundred (200) µl from already prepared 0.25 % MP's solution to PCR tubes;
Remove the buffer
•Add two hundred (200) µl from 8 to 2 µg/ml of DAG(Donkey Anti-Goat)-Obelin conjugate in Reaction Buffer in duplicates and incubate 30 min at room temperature with shaking (maximum speed 10 on Lab Line instruments titer plate shaker);
•After 30 min. incubation at room temperature with shaking, wash with Washing Buffer twice in the tubes, transfer the beads to the white strips (Greiner Maximum Binding, flat bottom White*) and wash twice again,
•Add fifty (50) µl of washing buffer to each well
•Trigger bioluminescent reaction by injecting 50 µl Triggering buffer, the emitted light was detected employing Luminoscan Ascent Thermoelectron plate luminometer.
Buffers:

[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

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Commercialization

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Production

- Current capacity for silver plated beads - manufacturing commercial lots
- Customized beads might be produced
  - Beads conjugated to antibodies, affinity tags
  - Beads conjugated to universal tag
- Adjust to requirements of large customers
- Complete/partial third-party manufacturing

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Marketing

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Novel Paramagnetic Beads

Market advantage

- **Applications that would benefit from use:**
  - Increase test sensitivity by at least ~5-fold, early diagnosis
  - Easy sample manipulation
  - Custom-made linking chemistry

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Novel Paramagnetic Particles

Primary target markets

- Clinical In-Vitro-Diagnostics testing that employ
  - Traditional paramagnetic particles
  - Light signaling mechanism

- Life sciences research testing applications
- Novel markets - applications that would benefit from the silver layer/light amplification

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Potential Markets

- Clinical In-Vitro-Diagnostics testing that employ
  - Ten (10) first-tier global IVD test manufacturers
  - Thirty (30) second-tier IVD test manufacturers
  - Numerous small regional manufactures

Could be adapted to existing/new tests/machinery

- Life sciences research applications, companies/universities

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Commercialization

- Clinical In-Vitro-Diagnostics testing that employ
  - A development, manufacturing and marketing agreement signed January 2009 With Merck Chimie
  - CardioGenics royalty is 30% of sales, paid on quartiles basis

- Life sciences research applications, companies/universities
  Third party distribution
- Large customers may require third-party manufacturing
- Technology transfer (partial)/Process licenses under agreements

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Novel Paramagnetic Beads

Summary

We have developed paramagnetic particles that:

- Are light-colored
- Could be made in different sizes
- Several surface functional groups
- Hydrophilic polymer surface layer
- Stable from magnetic/chemical aspects
- Exhibit ~5-fold improvements in testing sensitivity
- Executed distribution agreement with Merck Chimie, for 10 years

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Thank you

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