MELISA® In-Vitro Methodology for the Detection of Metal Hypersensitivity in an American Population

Pranay D. Khare, Ph.D.[1], Mieke Kellermann[2], Gottfried H. Kellermann, Ph.D.[1, 2]

[1]NeuroScience, Inc., Osceola, WI USA, [2]Pharmasan Labs, Osceola, WI

Abstract

Background: Although heavy metals such as copper, iron and zinc are essential to maintain normal physiologic functions, many metals, including aluminum, mercury, lead, nickel, and titanium, among others, may be a cause of metal hypersensitivity, which can be a root cause of chronic inflammation and disease. Lymphocyte testing is more sensitive than dermal patch testing for metal hypersensitivities because the cellular response to dermal contact is often different than that in an *in-vivo* environment. Dermal patch testing may also induce allergies, and there are no standardized tests for dermal patch testing for all relevant metals. There is an urgent need for a functional *in-vitro* test that can determine heavy metal hypersensitivity in US population.

Objective: The aim of this study was to evaluate the reproducibility, sensitivity, specificity and reliability of an *in-vitro* MELISA® (Memory Lymphocyte Immuno-Stimulation Assay) test for detecting type IV hypersensitivity to metals in a US population. Multiplex cytokine analysis was also performed in patients identified with metal hypersensitivity to assess inflammatory status.

Design: 525 MELISA® tests for metal hypersensitivity against aluminum, mercury, lead, methyl mercury, nickel, thimerosal, and titanium were performed on enriched lymphocytes obtained from the peripheral blood of 75 healthy US subjects. The frequency and distribution of reactivity, and the sensitivity and specificity of the assay were analyzed. In addition, 308 metal tests were performed to determine inter-assay variability and 116 metal tests were performed to evaluate intra-assay variability. Proficiency testing was performed in laboratories in the US and Geneva, Switzerland. Microscopic examination on 126 metal tests was performed for accuracy studies with lymphocyte reactivity. The effect of varied heavy metals on cytokine production was also examined using Multiplex technology.

Results: MELISA® testing identified 36% of asymptomatic subjects tested positive for metal hypersensitivity. Of these, 66.6% were identified with hypersensitivity to 1 metal, 25.9% to two metals, and 7.4% to three metals. The most frequent hypersensitivity was nickel (24%), followed by mercury (13.3%), lead (6.6%), titanium (2.6%), thimerosal (2.6%) and aluminum (1.3%). The accuracy of the MELISA® test was 99% using the cut-off of Stimulation index ≥3. Intra-assay variability was maximum with lead (18.9%), followed by titanium (16.1%), aluminum (14.1%), mercury (11.6%), thimerosal (11.2%), nickel (10.1%) and methyl mercury (5.3%). Proficiency testing for nickel, thimerosal and methyl mercury was 14.6%, 11.7% and 14.8%, respectively when data were compared between labs. Cytokine assessment on subjects with positive MELISA® test results indicated an activated immune system, producing high amounts of pro-inflammatory cytokines and chemokines (IL-1β, IL-2, IL-6, IL-8, IL-17 and MIP-1β).

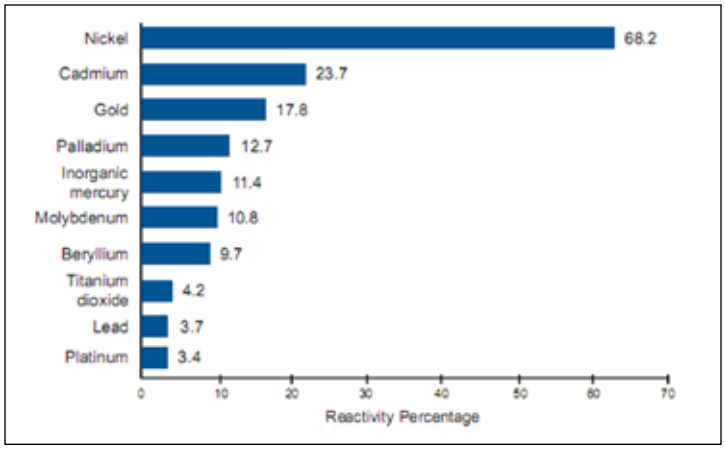
Conclusion: The MELISA® assay is highly reproducible, sensitive, specific, and reliable in detecting metal hypersensitivity in an American population. *In-vitro* MELISA® and Multiplex cytokine testing is clinically useful in patients with suspected metal hypersensitivity and chronic diseases. To our knowledge, this is the first comprehensive study that identifies metal hypersensitivity in an American population by MELISA® and the effect of heavy metal hypersensitivity on cytokine production.

Background Information

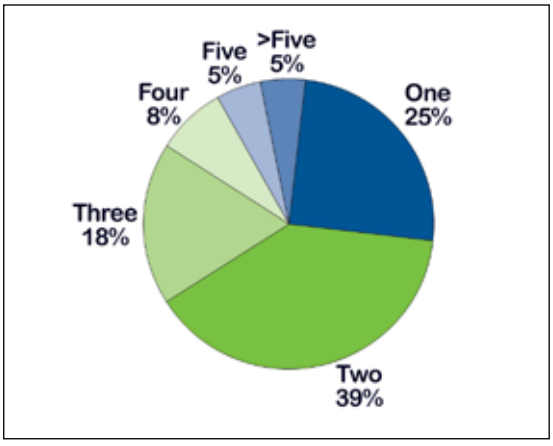
- Metal hypersensitivity is a reaction of the body's immune system against certain metals (1).
- T-lymphocytes play a crucial role in the induction of immune responses (2).
- The immune response induced by heavy metals can be verified by the presence of antigen-specific memory T-cells *in-vitro* (3).
- The MELISA® (Memory Lymphocyte Immuno-Stimulation Assay) is a validated technology to detect type IV hypersensitivity to heavy metals (4).
- The clinical relevance of MELISA® has been assessed for detecting and monitoring hypersensitivity to metals (5).

Metal Sensitivity in Symptomatic Population (n=700)

Frequency[1]



Prevalence[1]

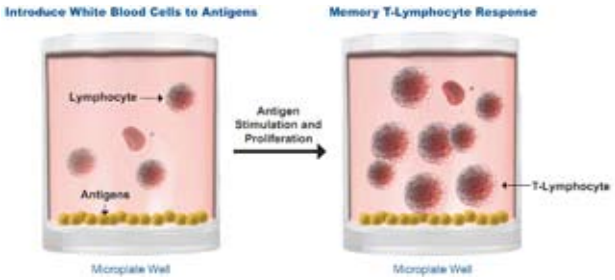


Objective

To evaluate the reproducibility, sensitivity, specificity and reliability of an *in-vitro* MELISA® (MEmory Lymphocyte Immuno-Stimulation Assay) test for detecting type IV hypersensitivity to heavy metals in a U.S. population.

Methods

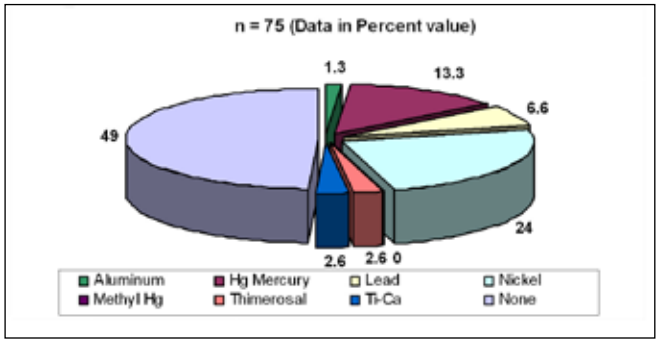
- Whole Blood Collected from healthy US population (n = 75).
- Peripheral blood mononuclear cells (PBMCs) isolation by Ficoll, wash 2X
- PBMCs culture enriched for lymphocytes, exposed to 24-well plates pre-coated with metal solutions, Incubate for 5 days at 37°C, 5% CO_2
- Cells pulsed for 4 hours with ^{3}H-thymidine, Harvest cells and measure radioactivity (cpm)
- Calculate Stimulation Index (SI) = cpm in test well / average cpm in negative control wells
- Calculation: SI < 2: negative; SI between 2-3: equivocal; SI >3 positive
- Additional morphological analysis for lymphoblast in positive and equivocal
- Metal Tested: Nickel, Inorganic Mercury, Methyl Mercury, Lead, Titanium, Thimerosol, Aluminum



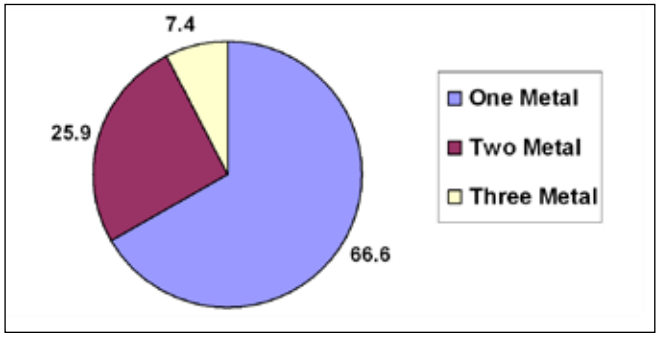
- Cytokine Determination: Multiplex human-Cytokine Assay (Bio-Rad).

Results

Frequency of Metal Hypersensitivity in Asymptomatic American Population



Distribution of Metal Hypersensitivity in Asymptomatic American Population



Results

Accuracy of MELISA® Test

Aluminum Thymidine (SI)	Aluminum Microscopy	Hg Mercury Thymidine (SI)	Hg Mercury Microscopy	Lead Thymidine (SI)	Lead Microscopy	Nickel Thymidine (SI)	Nickel Microscopy
0.8	-	1.1	-	0.8	-	0.8	-
1.1	-	1.8	-	1.2	-	6.1	+++
2.3	+/-	1.9	-	6.0	+++	1.5	-
1.0	-	5.1	+++	7.9	++++	1.6	-
0.7	-	1.1	-	2.4	+/-	0.4	-
0.4	-	0.6	-	0.9	-	18.9	+++++
1.2	-	4.1	+++	3.1	+	8.2	++++
0.9	-	5.3	+++	1.6	-	1.1	-
0.9	-	1.1	-	0.9	-	1.7	-
1.0	-	11.4	++++	2.6	+/-	3.8	+
0.9	-	3.1	+	1.5	-	7.1	++++
6.3	+++			1.1	-	1.5	-
0.7	-	5.8	+++	0.7	-	0.9	-
0.9	-	1.7	-	0.8	-	3.7	+
0.7	-	1.4	-	1.7	-	8.7	++++
0.9	-	4.4	+++	1.1	-	5.7	+++
0.6	-	2.5	+/-	1.9	-	0.8	-
1.2	-	2.3	+/-	1.6	-	59.5	+++++

Result: The accuracy of MELISA® test was 99% with Morphological Observations.

Intra-assay Variability of MELISA® Test

Hg	Mean	SD	%CV
	2.10	0.43	20.4
	1.10	0.13	11.7
	1.30	0.17	13.2
	1.00	0.01	1.0
	0.80	0.03	3.7
	1.60	0.33	20.6
Mean %CV			11.8

Lead	Mean	SD	%CV
	2.20	0.4	19.30
	1.10	0.5	40.70
	1.30	0.4	27.30
	0.80	0.1	9.60
	3.30	0.1	2.00
	3.40	0.5	16.10
Mean %CV			19.2

Titanium	Mean	SD	%CV
	1.2	0.02	1.5
	0.6	0.2	33
	1.2	0.2	14.2
Mean %CV			16.2

Thimerosal	Mean	SD	%CV
	0.5	0.04	8.0
	0.4	0.1	18.1
	1.1	0.1	7.4
Mean %CV			11.2

Result: Intra-assay variability was 19.2% for lead followed by titanium (16.2%), aluminum* (14.1%), mercury (11.8%), thimerosal (11.2%), nickel* (10.1%) and methyl mercury*, (5.3%).

*Data not shown

Proficiency Testing of MELISA® Test

	Nickel	Methyl Hg	Thimerosal
In Geneva lab	1.5	0.7	0.8
Pharmasan Labs	1.2	0.5	0.9
Mean	1.35	0.6	0.85
SD	0.2	0.1	0.1
%CV	14.8	16.6	11.7

Result: The inter-assay variability of MELISA® test for nickel, methyl mercury, and thimerosal was 14.8%, 16.6% and 11.7%, respectively when data was compared between labs.

Results

Cytokine assessment on subjects with positive MELISA® test

Cytokines ↓	Metals →	Ni	Au	Ni	Ti
	Control	Patient 1	Patient 1	Patient 2	Patient 2
IL-1β	3.1	5.9	3.5	14.9	2561.9
IL-2	3.4	5.9	7	n.d.*	n.d.*
IL-6	94.5	460.6	391.6	118.1	13052.3
IL-8	3083	21303.4	26358.9	2076.7	36966.1
IL-10	0.3	0.4	0.4	0.2	113.8
IL-17	7.6	12.2	8.8	n.d.*	n.d.*
G-CSF	1.5	11.6	9.6	0.2	492.5
GM-CSF	5.8	11.2	10.2	0.2	0.2
IFN-γ	20.7	54.4	36.5	0.2	0.2
TNF-α	1.6	4.3	3.8	0.2	1795

(Cytokine Concentration in pg/mL) ***n.d. = not determined**

Positive Cytokine results are red.

Conclusion

Our study concludes that:

- Metal hypersensitivity is highly prevalent in American population.
- The MELISA® assay is a highly validated technology and useful for detecting metal hypersensitivity in suspected patients.
- The MELISA® assay is highly reproducible, sensitive, specific, and reliable in detecting metal hypersensitivity in an American population.
- The MELISA® and multiplex cytokine assay is clinical relevant technology to determine the low grade inflammation in patients.
- Combination of *in-vitro* MELISA® and Multiplex cytokine testing is a highly specific tool to determine clinical metal hypersensitivity and associated chronic diseases.

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Contact Information

NeuroScience, Inc.
Attn: Pranay D. Khare, PhD
375 280th Street
Osceola, WI 54020
Email: pranay.khare@neurorelief.com

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