



DEPARTMENT OF ORTHOPEDIC SURGERY
 Nadim J. Hallab, Ph.D.

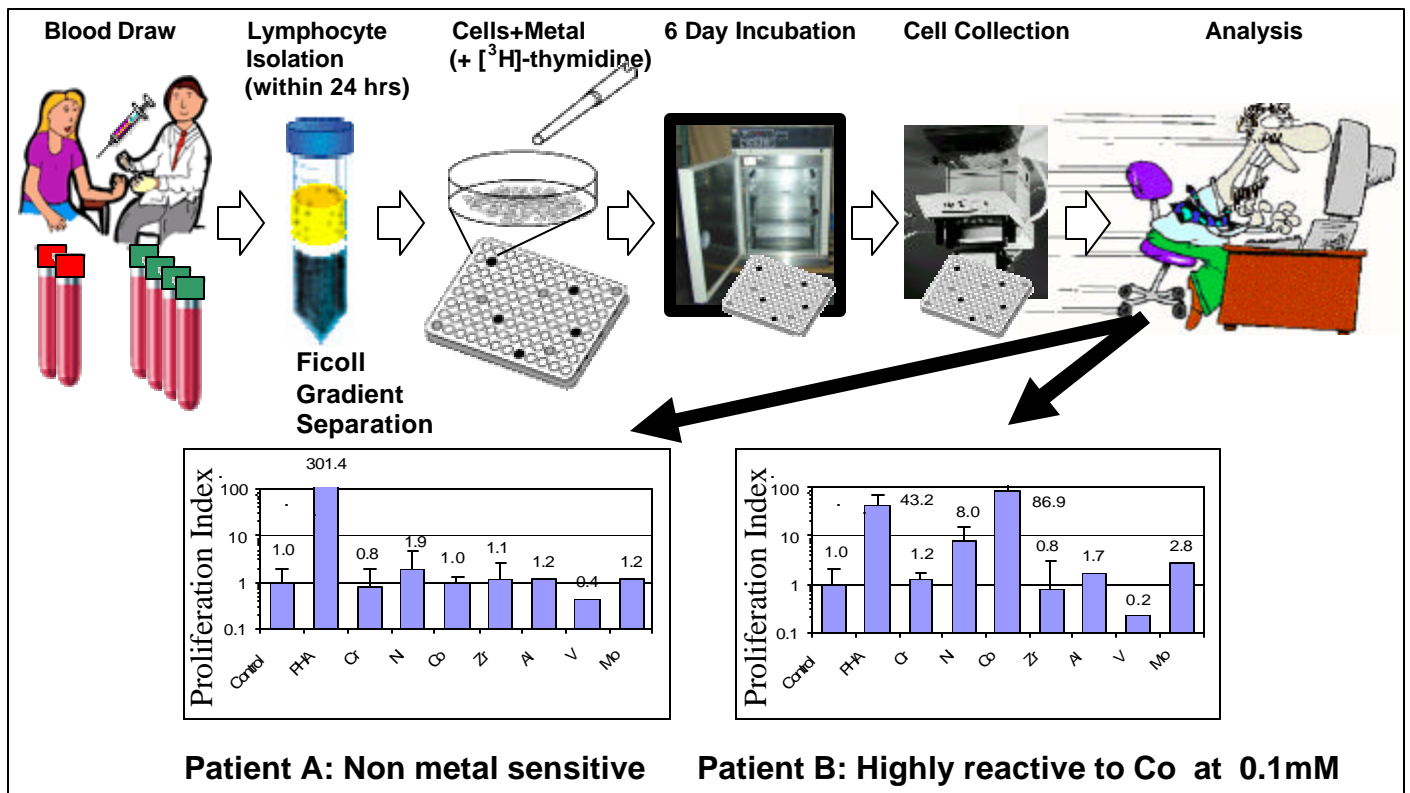
IN-VITRO METAL HYPERSENSITIVITY TESTING - ASSAY METHODOLOGY

The clinical observations of unexplained pain, effusion, stiffness and/or cutaneous eruptions following total joint arthroplasty have increased the need for a more accurate methodology for diagnosing "metal allergy" (hypersensitivity) to metallic biomaterials. Current methods used to diagnose hypersensitivity reactions, such as dermal patch testing, are not well accepted in orthopedic practice, and involve the possibility of inducing hypersensitivity responses. To address this need we have developed an *in-vitro* LTT assay to test for metal sensitivity to Aluminum, Chromium, Cobalt, Molybdenum, Nickel, Vanadium, and Zirconium.

Proliferation Assays (Lymphocyte Transformation Tests): Lymphocytes cultured for 1 week in the presence of metals (0.01, 0.1 and 1.0mM Al, Co, Cr, Mo, Ni, V and Zr) and measured for a response. **Interpretation:** This assay facilitates a dose response quantification of metal-induced hypersensitivity responses in terms of generalized lymphocyte reactivity (i.e. proliferation). Issues of sensitivity and specificity remain unresolved as well as how implant performance is related to positive reactivity results. Metal-specific reactivity is and gauged by comparing to non-treated to treated lymphocytes from the same individual and categorized using the general criteria: 24 fold =mild reactivity, 58 fold =moderate reactivity, and >8 =high reactivity.

Proliferation Assay (Lymphocyte Transformation Tests): Proliferation of cells is measured by [³H]-thymidine (Amersham International, Arlington Heights, IL) incorporation into DNA in a 96-well microplate system. The average for each treatment is normalized to that of the negative control (no treatment) producing a ratio, generally termed a proliferation factor, proliferation index, proliferation ratio or stimulation index, SI. The SI is used to compare lymphocyte reactivity to the different metals. The lower limit of this stimulation index is zero indicating all cells stopped dividing before addition of [³H]-thymidine, after 5 ½ days. Proliferation assays are performed using Ficoll separated mononuclear/lymphocyte cell fractions collected from 30 milliliters of peripheral blood per patient. These lymphocytes are cultured in 96-well cell-culture plates (Sigma), at a density of 0.1-0.3x10⁶ cells/well for a period of 6 days in 150µL of DMEM/well, 10% autologous serum at 37° C and 0.5% CO₂, with metal treatments, a positive control (0.01 mg/ml PHA) and a negative control (untreated). Each treatment is conducted in triplicate (3 wells/treatment). [³H]-thymidine is added during the last 12 hours of incubation after 5 ½ days of treatment. At day six [³H]-thymidine uptake (1 mCi/culture well) is measured using liquid scintillation. The SI is calculated using measured radiation counts per minute (cpm): $Simulation\ Index = (mean\ cpm\ with\ treatment) / (mean\ cpm\ without\ treatment)$.

Six days of incubation are chosen to reproduce, *in vitro*, the time lag associated with *in vivo* lymphocyte proliferation in a DTH response. Radiolabeled lymphocytes are collected onto membranes using a cell harvester (Tomtech Mach 2, Orange, CT) and the amount of differential radiation incorporation is measured using liquid scintillation (Wallac 1205 Betaplate, Gathersburg, MD). Stimulation indices of 2-4 indicate mild reactivity, 5-8 moderate reactivity and above 8 high reactivity to metals.



REQUIREMENTS:

Basic requirements for testing include:

- 1) Signed consent and questionnaire forms (forms will be provided),
- 2) Fill all vacutainers provided (all collection apparatus including vacutainer tubes provided in kit),
- 3) Blood must be received approximately 24 hours after blood draw (return via 24 hour Fed-ex), and
- 4) Donation to the Department of Orthopedic Surgery, Rush-Presbyterian-St. Lukes Medical Center (approximately \$250 to cover material costs only).

The results are generally available within 10 business days following the blood draw (1 week of cell culture is required for testing).

IMPORTANT DISCLAIMER

These lymphocyte reactivity *in vitro* assays have not been established as standardized clinical tests and have not been approved for diagnostic purposes by any institutional or government agency. The results of this testing must be carefully evaluated by a physician. A negative result for metal hypersensitivity does not necessarily prove that metal hypersensitivity does not exist; a positive result does not necessarily prove its presence. It is uncertain to what extent metal hypersensitivity mediates the pathogenesis of implant failure or other adverse reactions following total joint arthroplasty.

For more information please contact.

Tiffany Stafford: Metal Sensitivity Study Coordinator
Department of Orthopedic Surgery
747F Cohn Research Bldg.
1753 W Congress Parkway
Chicago, IL 60612
Tel (312) 942-5389

or

Dr Nadim Hallab
Department of Orthopedic Surgery
Rush-Presbyterian-St. Lukes Medical Center
1653 W Congress Parkway
Chicago, IL 60612

Tel (312) 942-7079
FAX (312) 942-8828
Email: nhallab@rush.edu

Address to Fed-ex Blood

Dr Nadim Hallab (Metal Hypersensitivity Study)
Department of Orthopedic Surgery
747F Cohn Research Bldg.
1753 W Congress Parkway
Chicago, IL 60612