Rapid Automated Analysis of Lipoproteins in Blood by NMR

(Developed at NC State Univ and LipoScience)

David R. Morgan, PhD
NMR Service, LLC
North Carolina State Study Abroad Program

Contents of Today's Talk

- Introduction to Lipoproteins
- Collecting the NMR data
- Automating the whole process

History of Lipid and Lipoprotein Testing

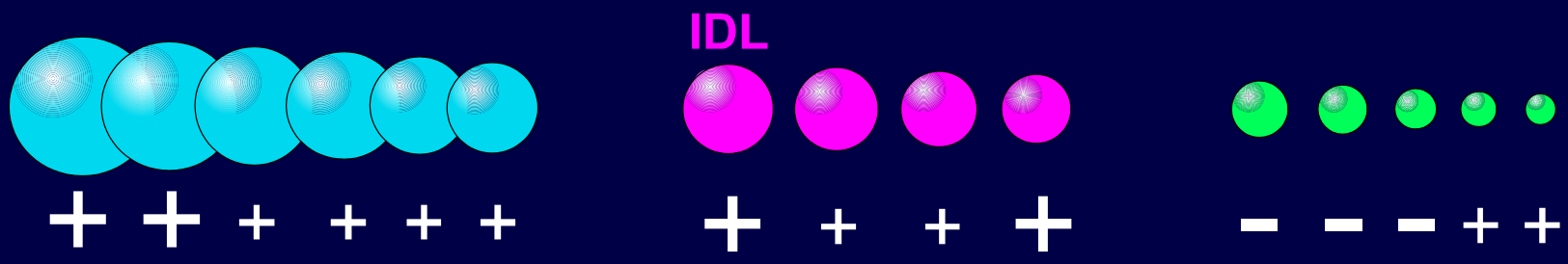
Total Cholesterol (+)

Fractionation (minutes)

VLDL-C + **LDL-C** + **HDL-C**

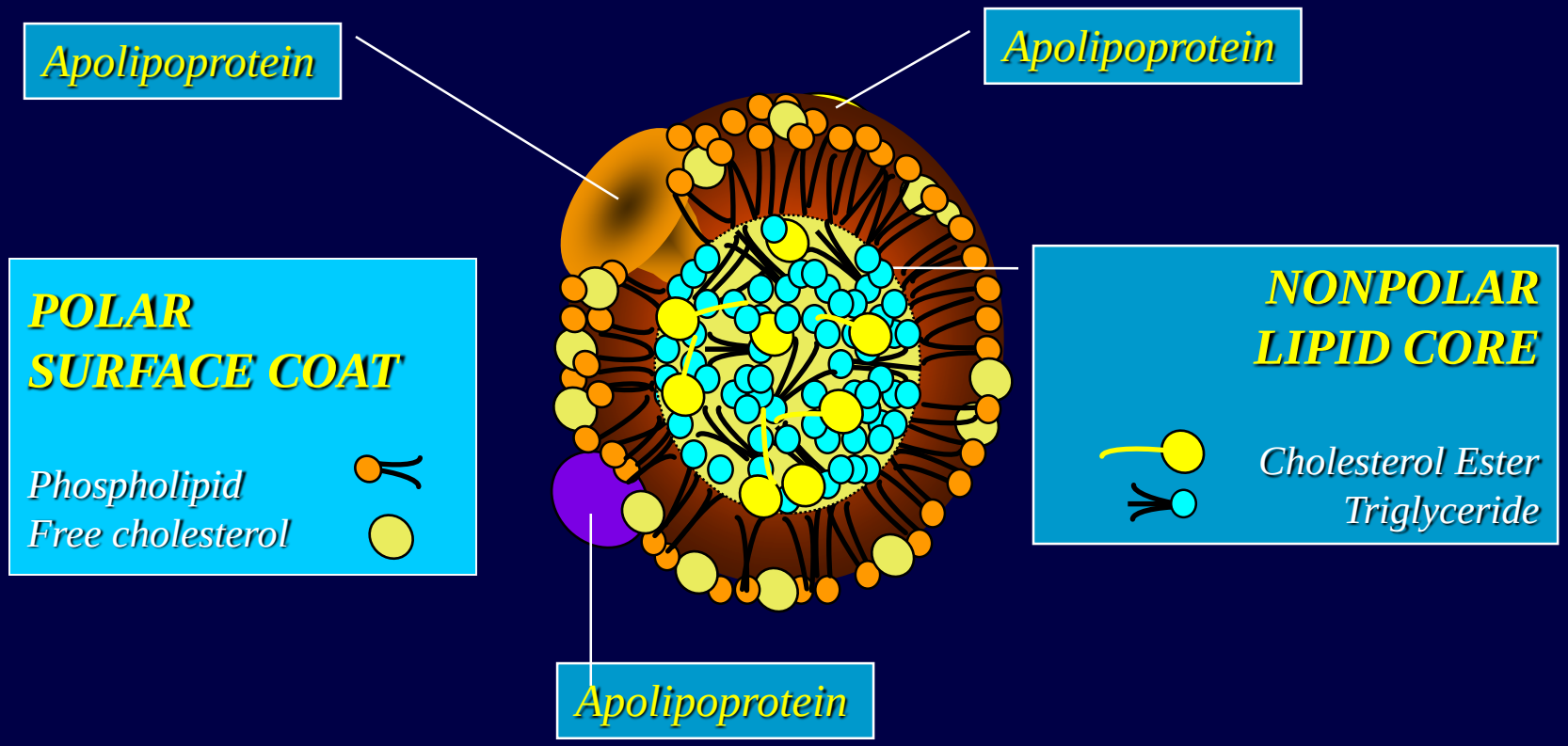
(+) (+) (-)

Subfractionation (many hours)

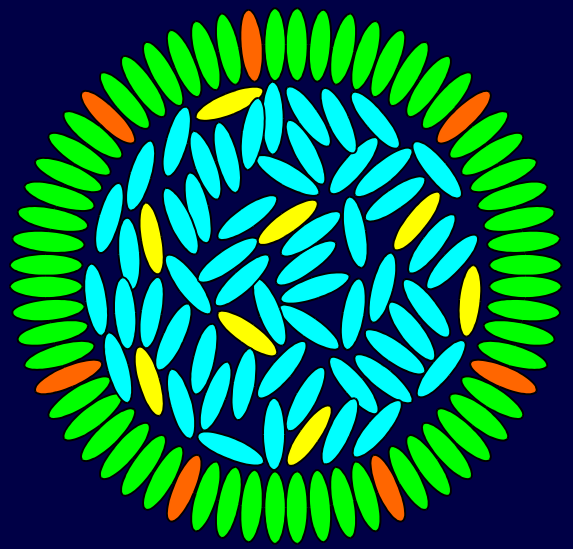


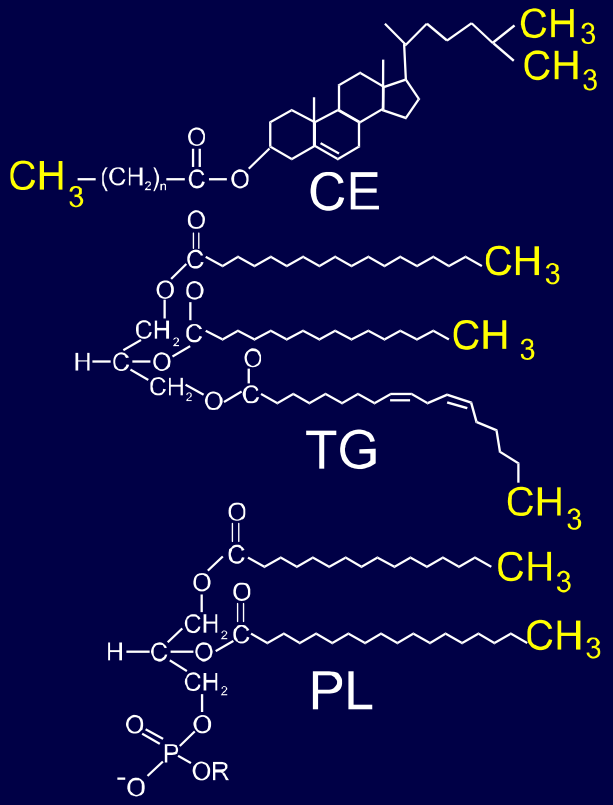
Association with CHD: Positive (+) or Negative (-)

Lipoprotein Particle Structure (VLDL, LDL, HDL)



The measured subclass signals come from the terminal methyl groups on the lipids in the particle shell and core





To a close approximation, the number of these methyl groups in a particle of given size is unaffected by lipid compositional variation. The measured amplitudes of the subclass signals are thus proportional to particle concentration.

Clin Lab Med 2006;26:847-70

A Natural Physical-Chemical Phenomenon Links Lipoprotein Diameters to NMR Signal Frequencies

VOLUME 72, NUMBER 25 PHYSICAL REVIEW LETTERS 20 JUNE 1994

Effects of Orientational Order and Particle Size on the NMR Line Positions of Lipoproteins

J. Lounila,* M. Ala-Korpela, and J. Jokisaari
Department of Physics, University of Oulu, FIN-90570 Oulu, Finland

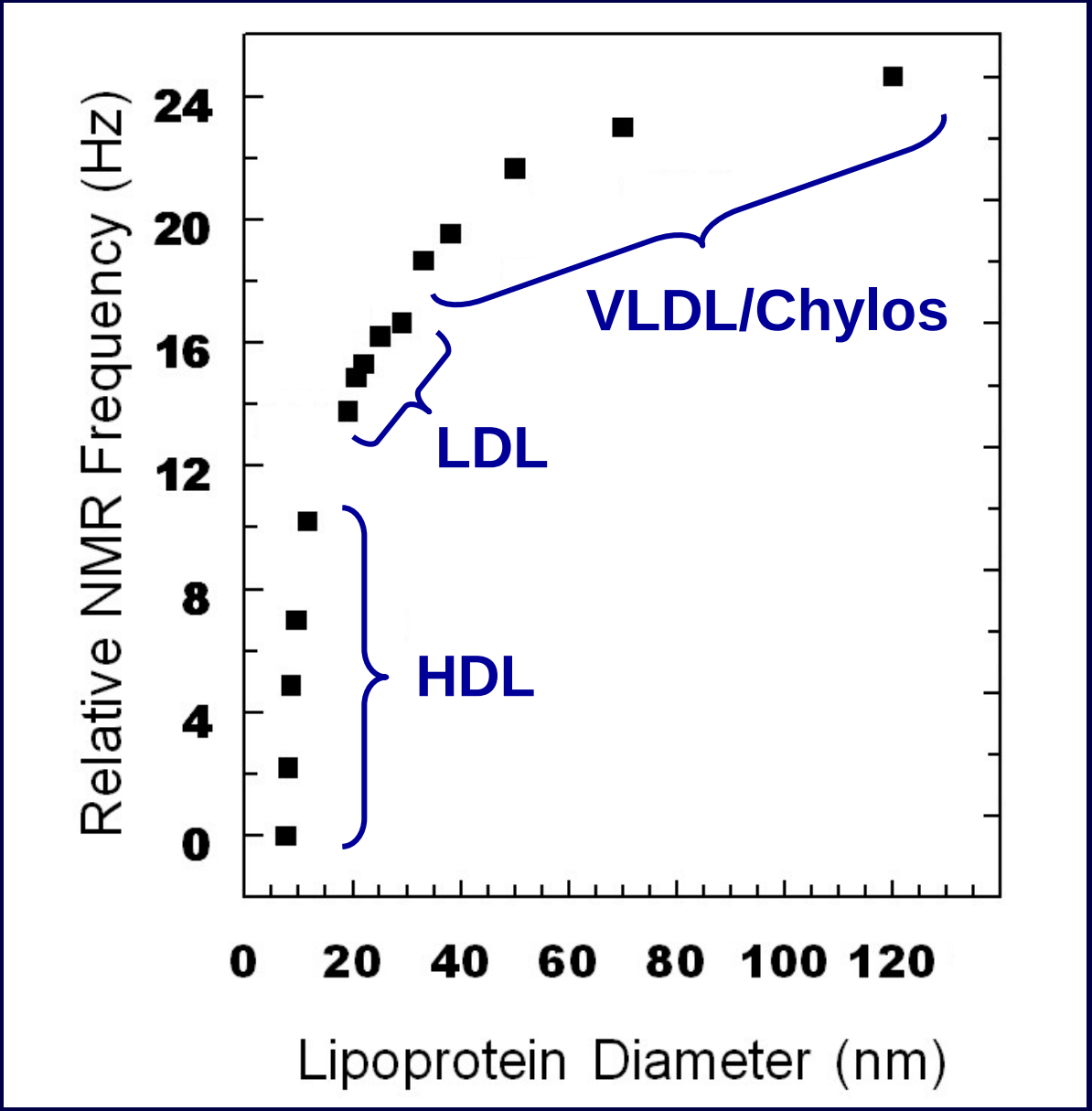
M. J. Savolainen and Y. A. Kesäniemi
Department of Internal Medicine and Biocenter Oulu, University of Oulu, FIN-90220 Oulu, Finland
(Received 24 January 1994)

We demonstrate that information on internal orientational order and size of lipoprotein particles can be extracted from the positions of their NMR spectral lines. The magnetic field obtained by solving the field equations for a model lipoprotein particle is shown to account for the hitherto unexplained size dependence of the experimental NMR frequencies. The predicted sign, magnitude, and functional form of the frequency shifts are verified by novel experimental ^{1}H NMR data from size-specific lipoprotein samples.

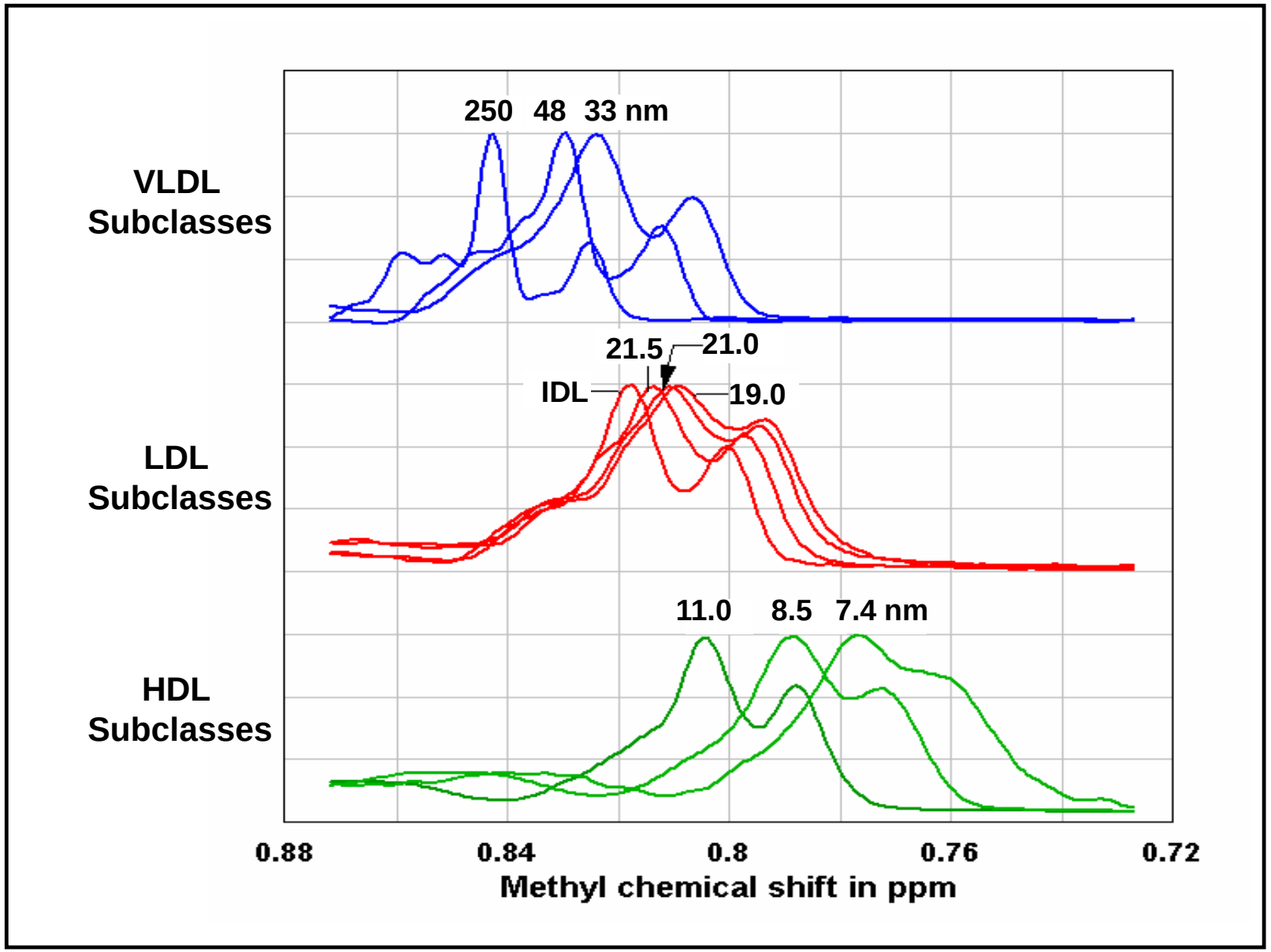
$$\mathbf{H}_i(r,\theta) = H_0\left[1 - a\chi_3 + A_i + \frac{2}{3}\Delta\chi_i \ln\frac{R_2}{r} + \frac{4}{9}\left(\frac{C_i}{r^3} - \Delta\chi_i\right)P_2(\cos\theta)\right]\mathbf{e}_\parallel + \frac{1}{3}H_0\left(\frac{C_i}{r^3} - \Delta\chi_i\right)\sin 2\theta\,\mathbf{e}_\perp ,$$

This <u>invariant</u> relationship makes lipoprotein subclass particles spectroscopically distinct, which enables their quantification without physical separation, chemical reactions, or sample pretreatment.

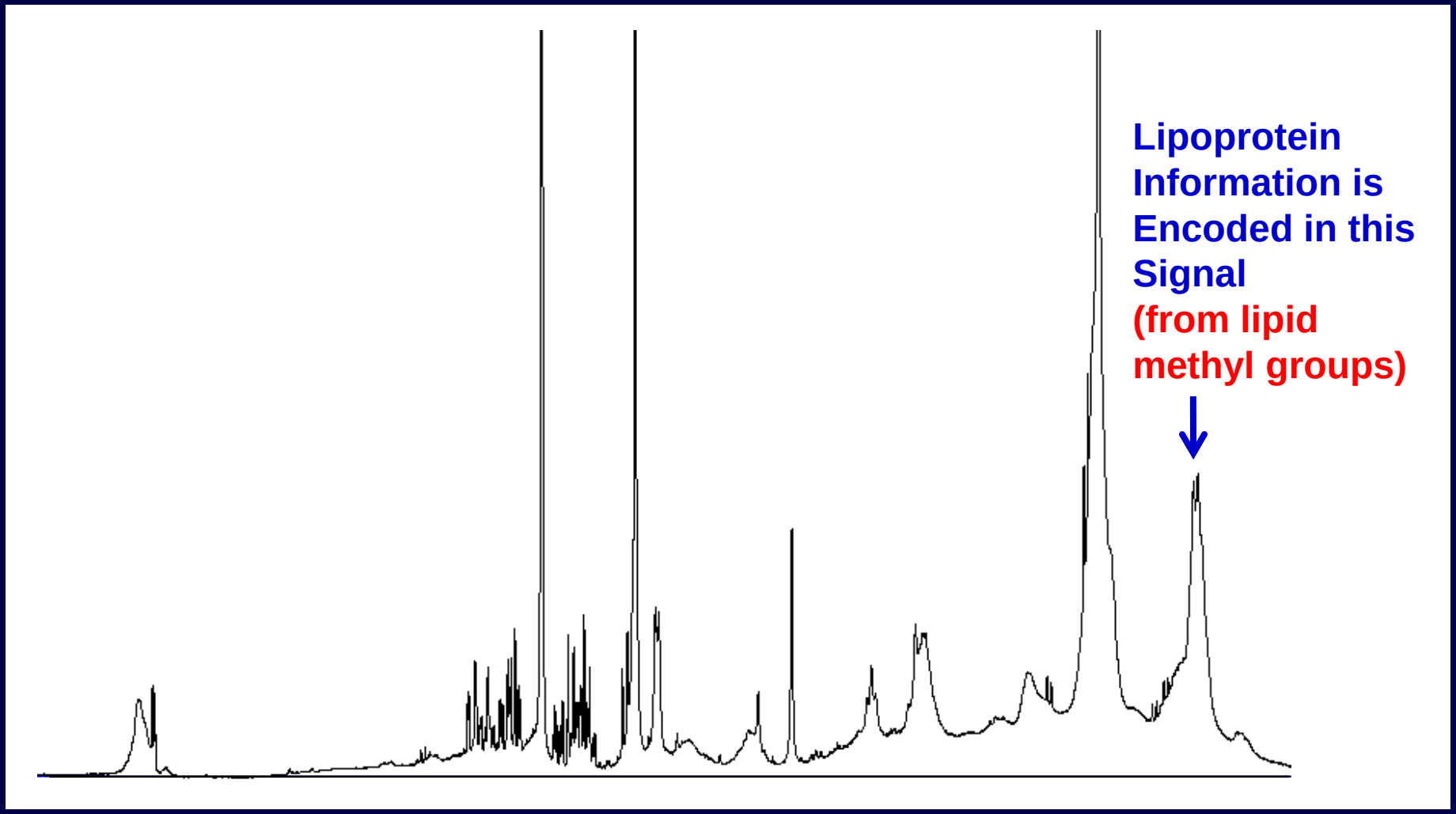
Particle Diameter Determines Lipid NMR Signal Frequency (and Lineshape)



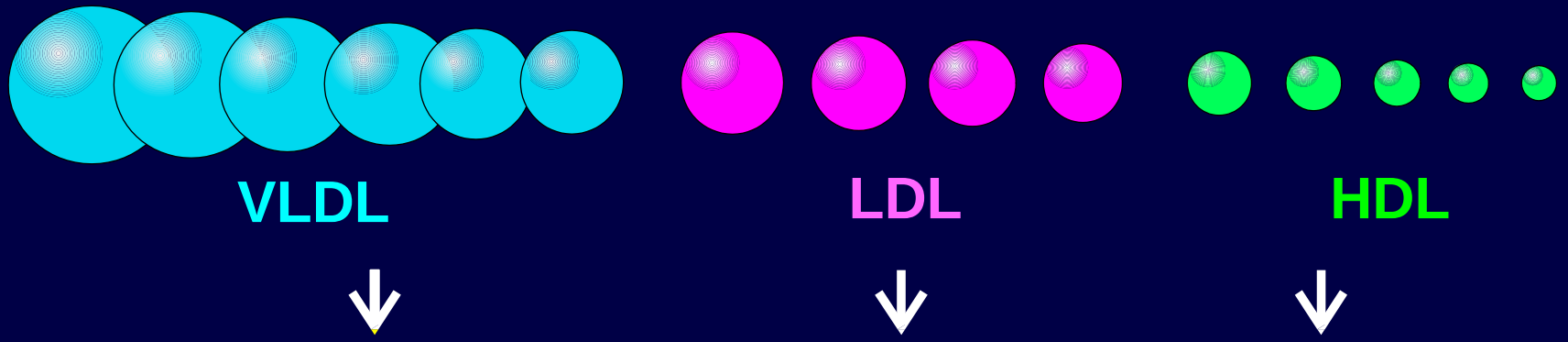
Lipid Methyl Group Signal from Isolated Subclasses



Proton NMR Spectrum of Blood Plasma

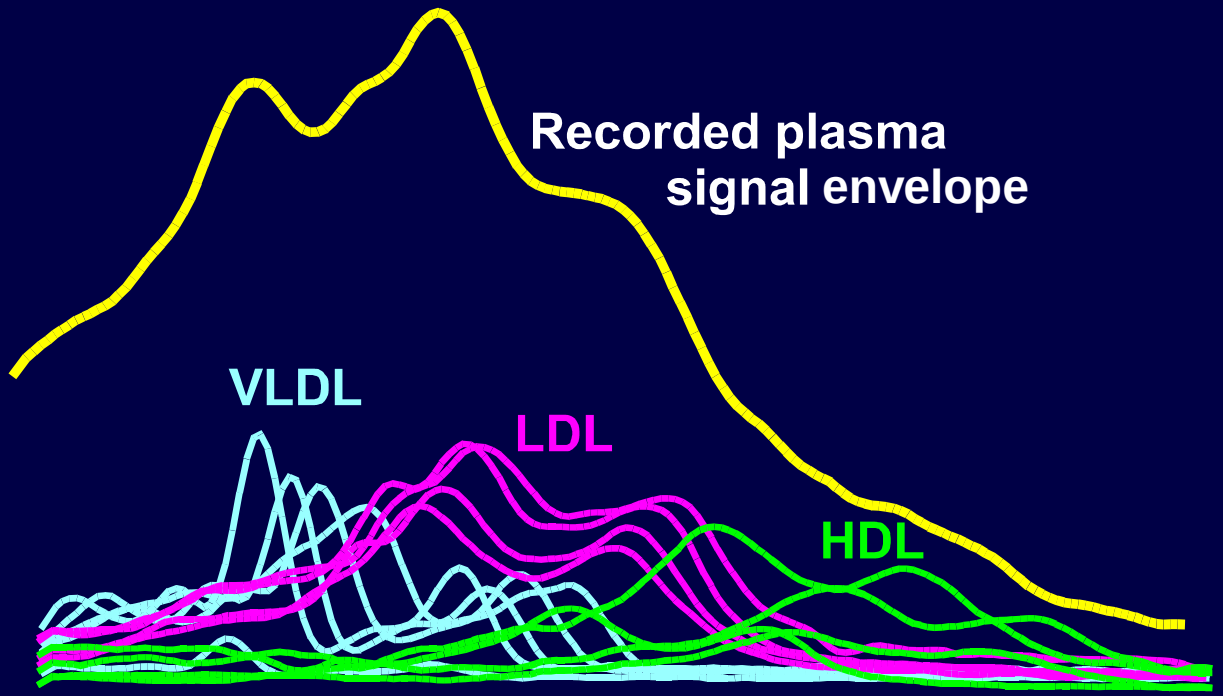


NMR Spectroscopy Measures VLDL, LDL, and HDL Particle Subclasses Simultaneously



"The whole is the sum of its parts"

The subclass signals combine to produce the measured plasma signal. The subclass signal amplitudes (derived by "deconvolution") give the subclass concentrations.



Clin Lab Med 2006;26:847-70

What makes NMR special?

- It is non-destructive
- You can do NMR on liquids and solids
- You can do NMR at high and low temperatures
- You can do NMR on mixtures- Metabolomics
- It is quantitative over a large range of concentrations
- It can give information on rates of change
- It can give you 3-dimensional structural information

Considerations in NMR Data Acquisition

Many variables must be considered:

- Magnet strength
- Pulse sequence
- Required S/N
- Processing parameters
- Glass tube or flow probe
- Addition of buffer and/or standards
- Temperature of sample

Acquisition of NMR Data

Many variables must be considered:

- Magnet strength - 9.4 T or 400 MHz
- Pulse sequence and acquisition parameters – WET water suppression, number of scans, etc.
- Required S/N - > 100
- Processing parameters – Resolution enhancement
- Glass tube or flow probe – Flow probe
- Addition of buffer and/or standards – Dilution buffer
- Temperature of sample – 47 C

Advantages of Flow NMR

- No need to buy expensive NMR tubes
- Simple waste disposal
- Flow cell never moves so the shims rarely need to change
- Permanent flow cell helps keep phase the same
- Volume of flow cell is constant which is important for absolute quantitation

Considerations for Using Plasma/Serum in Flow NMR

- ADVANTAGES
  - Consistent pH and ionic strength
  - Relatively high concentrations of analytes
- DISADVANTAGES
  - Viscous
  - Can have clots
  - Requires good cleaning protocol for tubing
-

Requirements For Rapid, Accurate Analysis

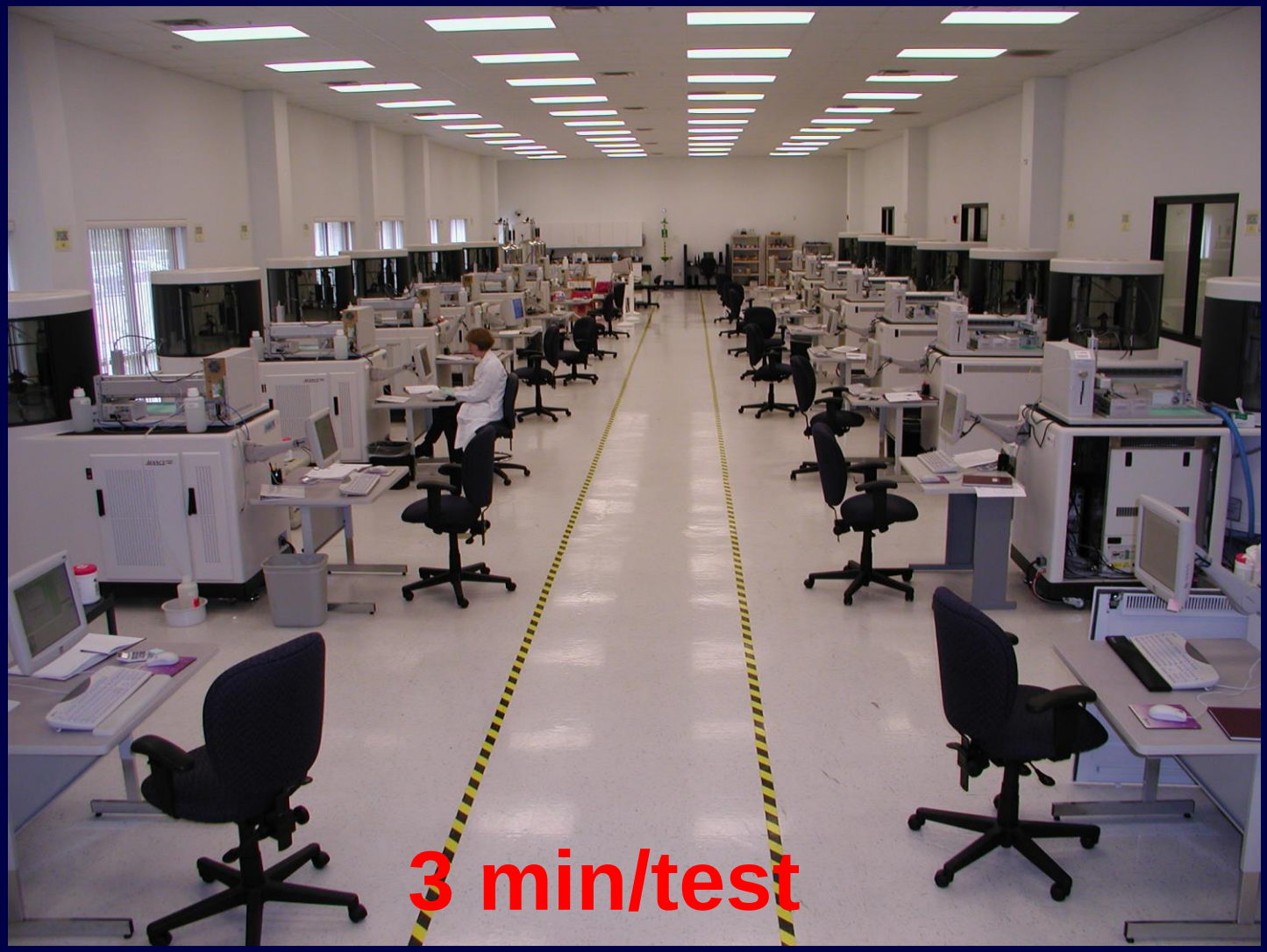
- Optimized fluidics, including heating the sample on the way to the magnet
- Optimized NMR parameters and calibrated NMR spectrometer. This means we don’t have to add an internal concentration standard!
- Integrated software and data processing

Steps for Acquiring a NMR LipoProfile

- Dilute the sample with buffer (Not an internal standard)
- Transfer the sample to the NMR probe
- Warm the sample to 47C
- Collect a water-suppressed proton NMR spectrum
- Apply a window function, Fourier transform the data and phase the spectrum
- Analyze spectrum with deconvolution software

Last Century Automation (1998)

2003 Centralized Testing in Raleigh, NC



We Learned a Lot From Running 15 Identical Instruments

- NMR Manufacturers don't have resources to thoroughly test their instruments-We became the experts on their systems
- We learned about instrument precision, instrument reliability, shim stability, magnet drift, vibrations
- We learned about human factors in doing repetitive, complicated tasks

After 4,000,000 samples it was time to do things differently
We needed to:

- Have the test run at multiple locations to save time and shipping costs
- Allow other labs to profit from running our test
- Improve the automation to make it easier and faster to run a test

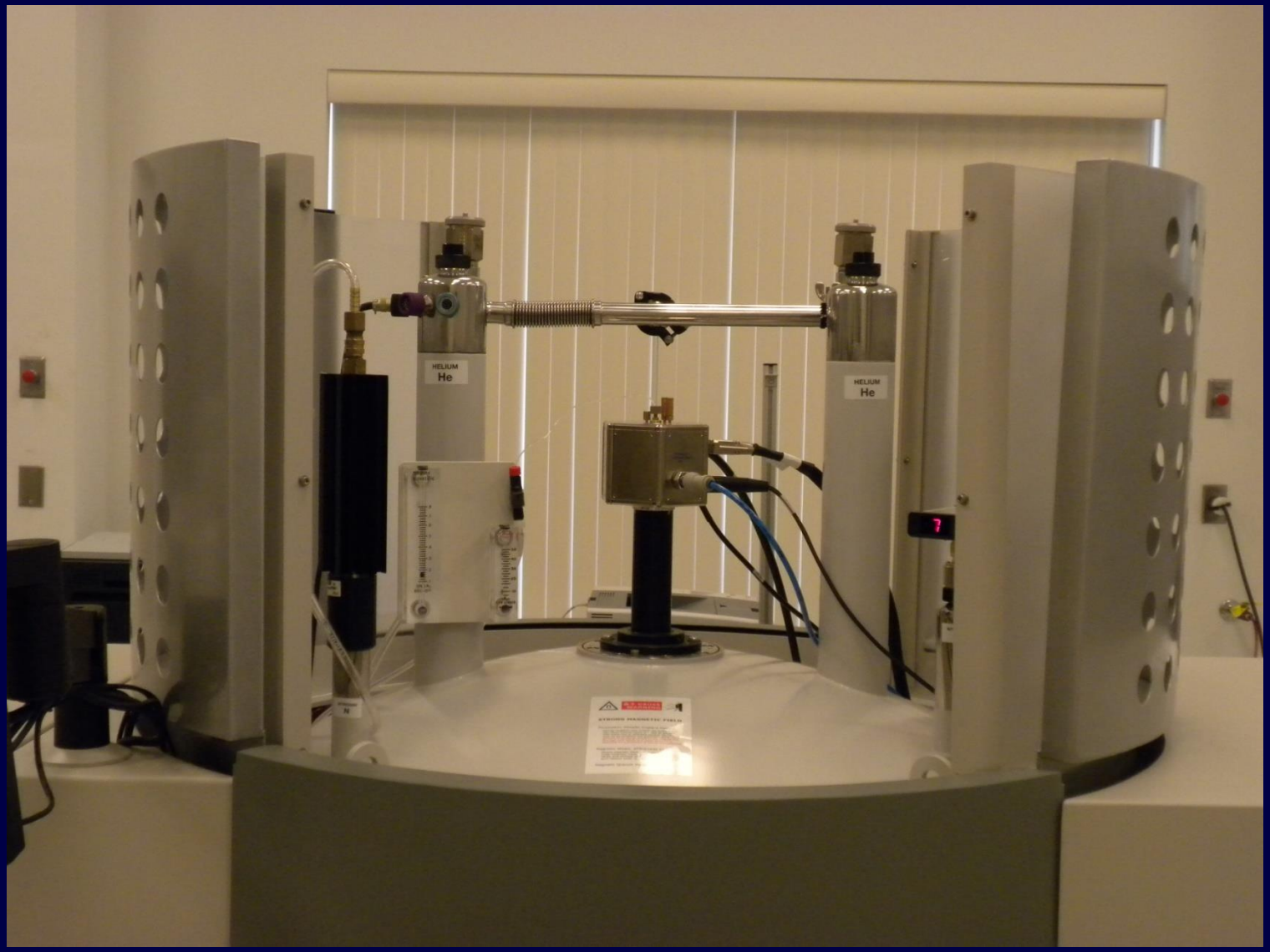
Design Goals For a New Instrument

- Look and feel like a typical clinical instrument
- Fluidics and NMR must be completely integrated
- Primary tube sampling, no pre-analytical processing
- Automatic calibration
- Reagent free, no kits or chemical reactants
- All processes including QC and final report are done on the instrument
- No knowledge of NMR required to run instrument

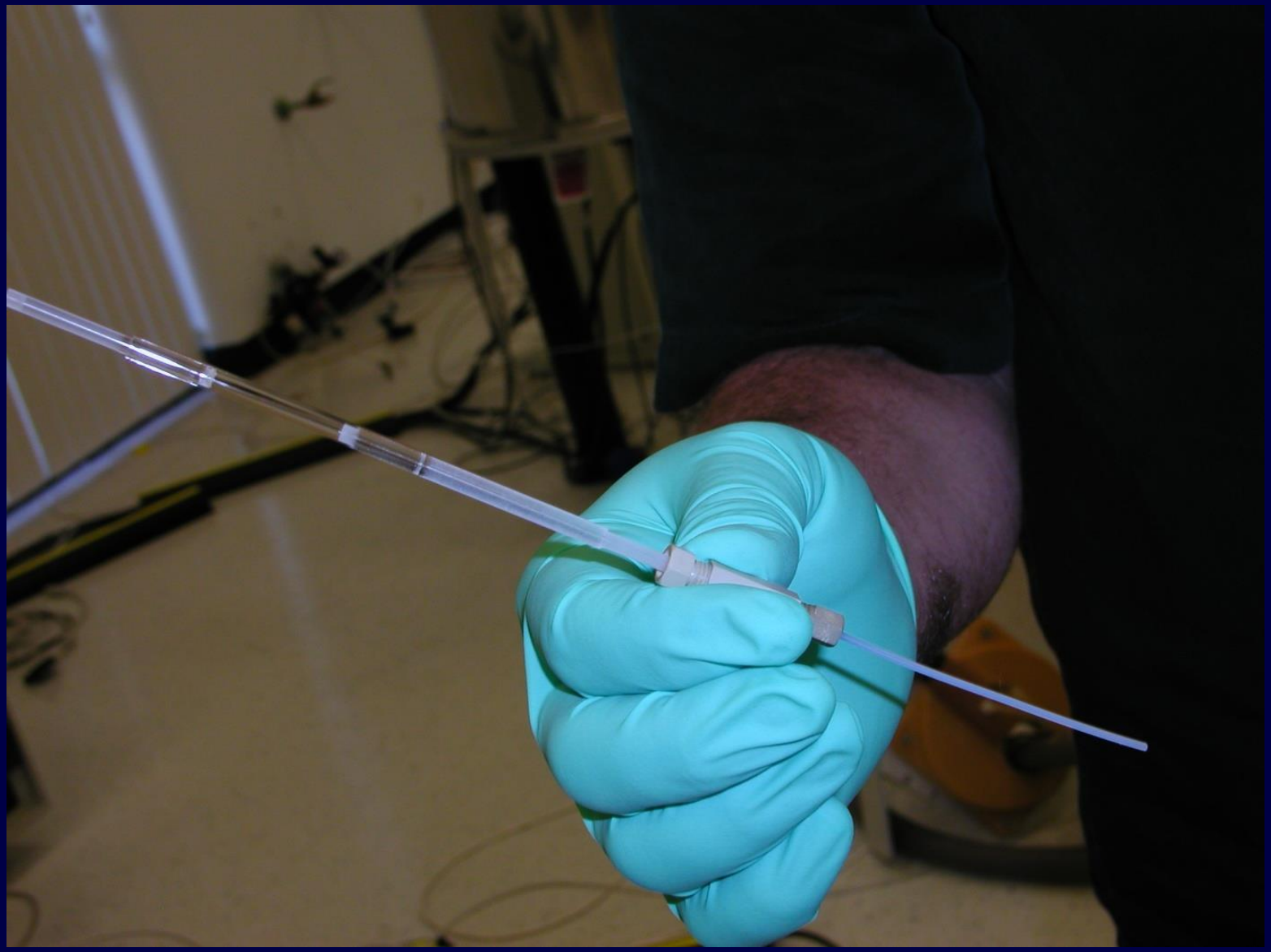
Prototype 3D Drawing

Reality

Magnet has been lowered and probe installs from the top



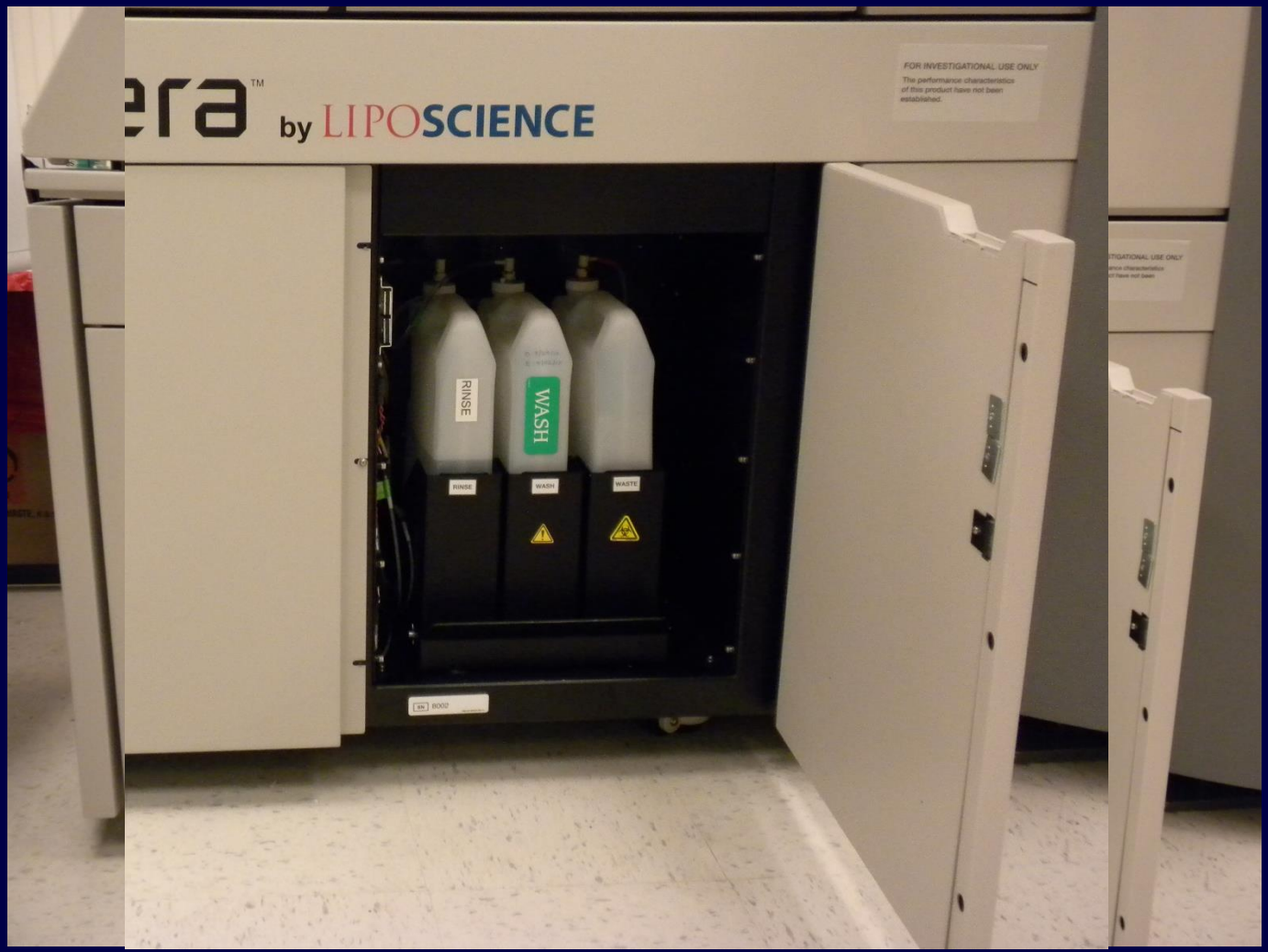
Replaceable flow cell



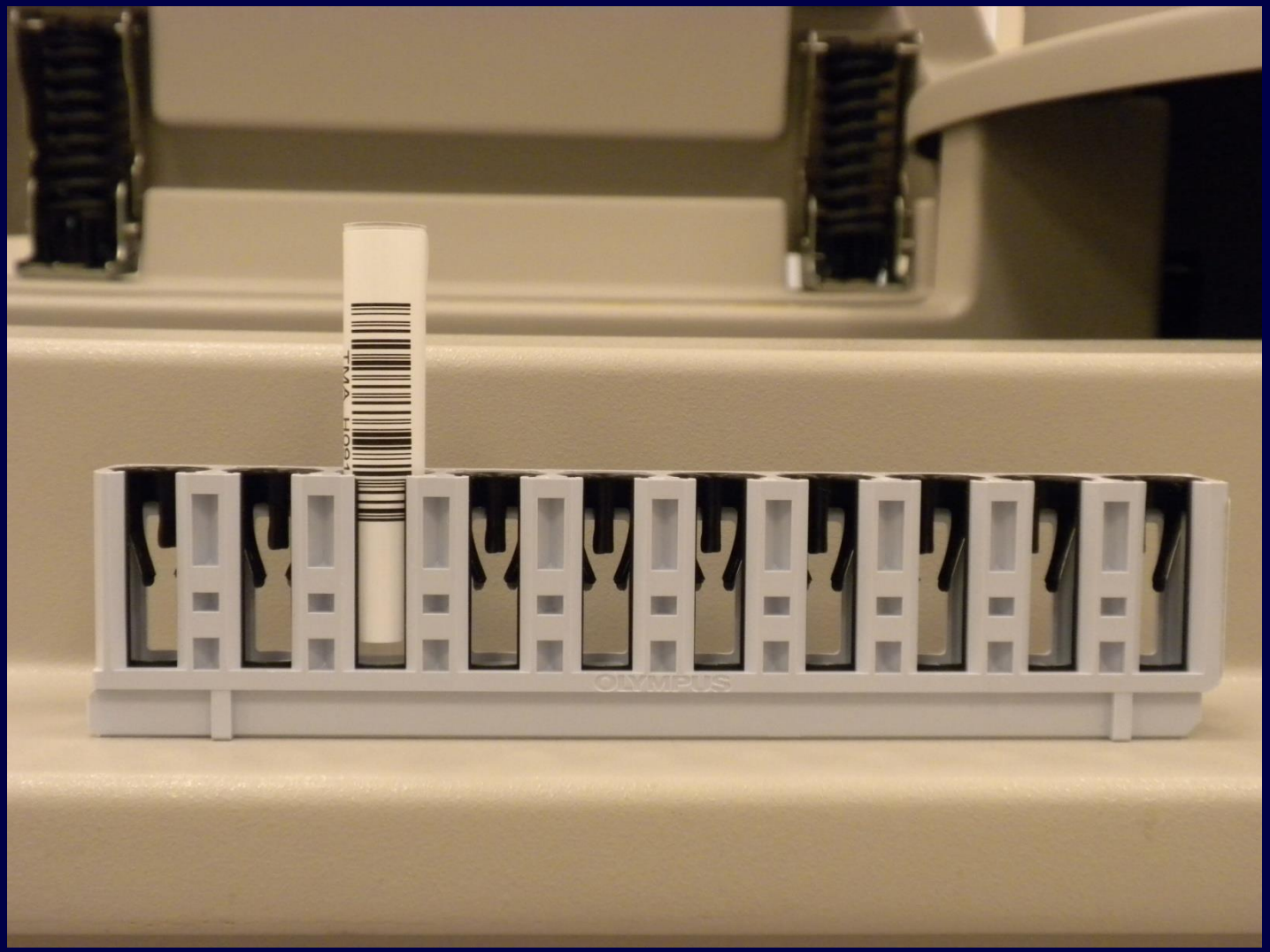
Standard Agilent 400 MR Console

On Board Bulk Fluids –

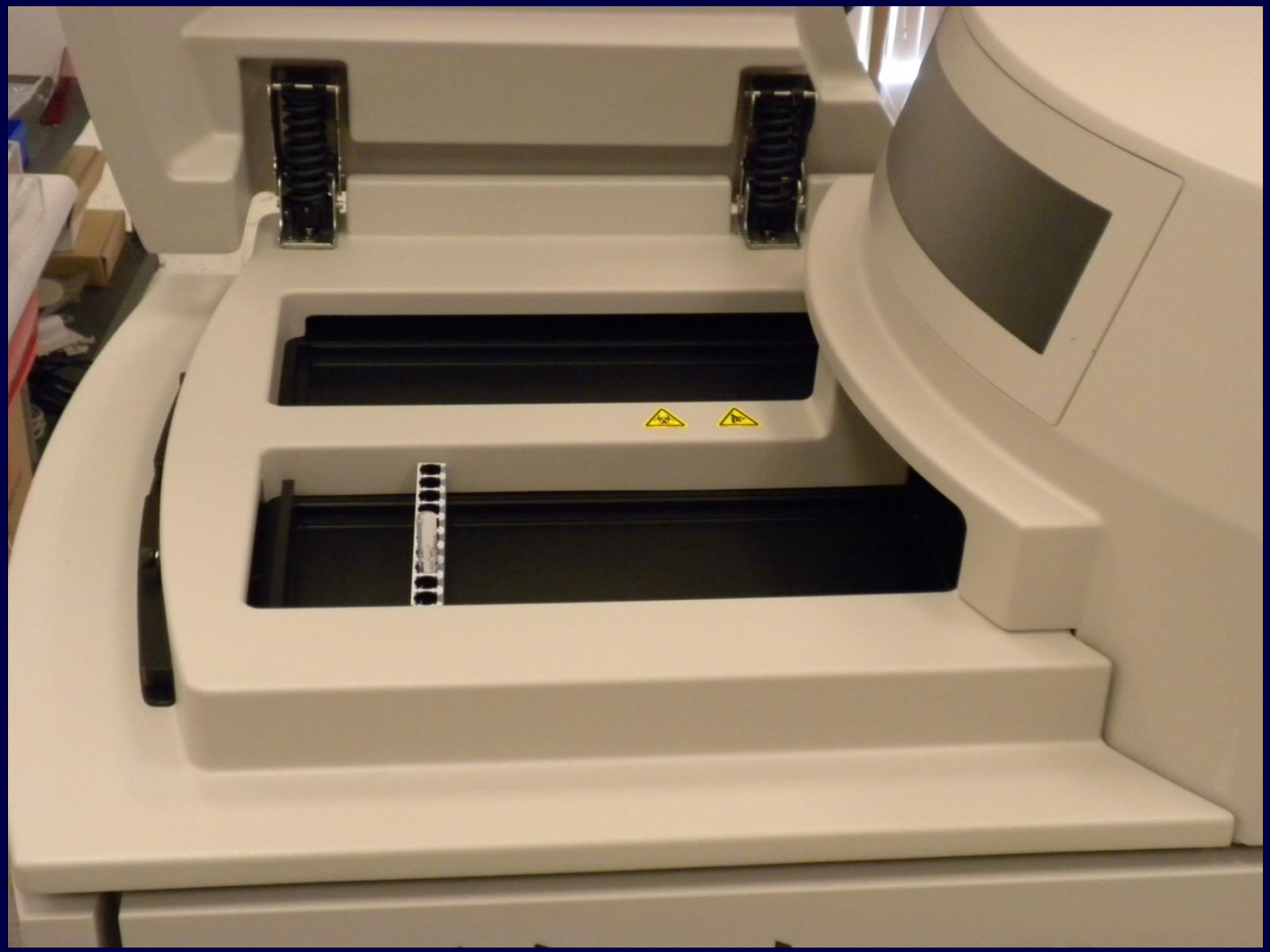
Wash, Rinse, Waste



Sample tubes fit on Olympus racks



Rack loading area. Up to 200 samples may be loaded at one time.



Prototype Testing Was Going Well Until One Warm Spring Day

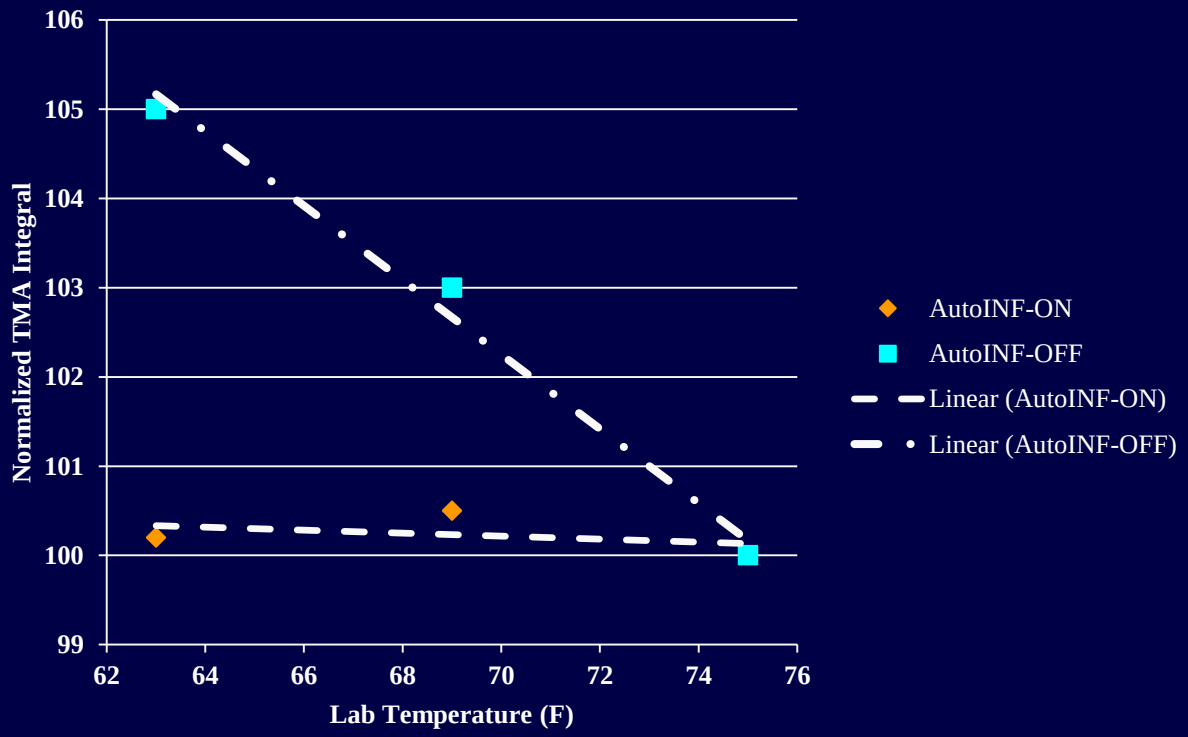
- We checked everything we could think of and finally realized it was related to the increased room temperature.
- What to do about it?

Temperature Chamber

Inside the Temperature Chamber

Temperature Problem Solved

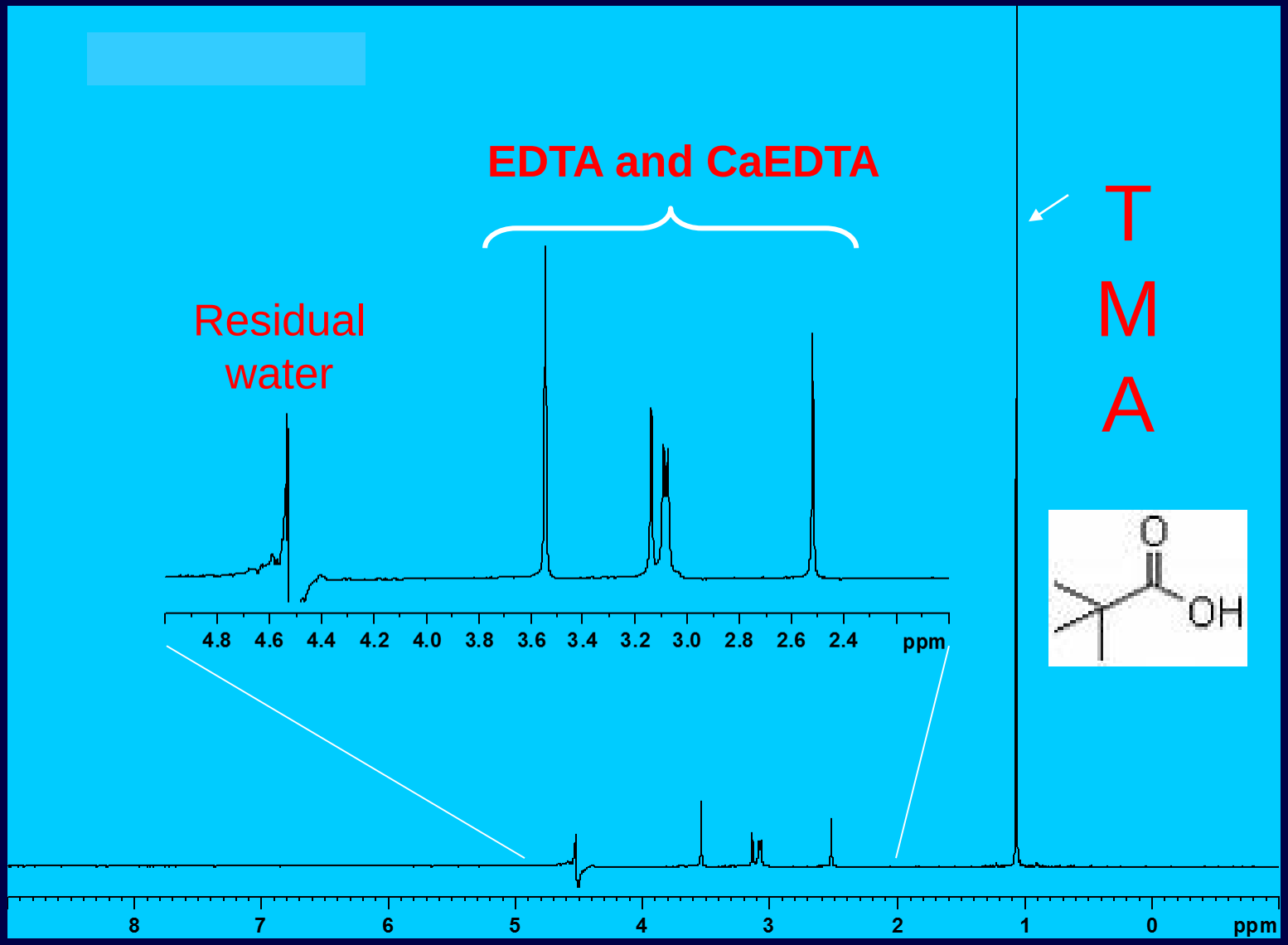
We added a temperature sensor to electronics to compensate for changes in room temperature



We Needed to Create a Standard Sample That Could Test the Instrument Performance

- It had to be easy to make
- It had to have a long shelf life
- It had to have similar NMR properties to serum/plasma
- Ideally, it would be cheap and have low toxicity

Spectrum of NMR Reference Standard



What Information Does the NMR Reference Standard Provide?

- Shows that the NMR is working
- Allows us to shim the magnet
- Allows a day-to-day comparison of system sensitivity
- Allows us to measure the temperature of the sample
- The sharp peaks and flat baseline allow us to monitor vibrations and measure the signal/noise
- Allows us to find the phase
- We can check the pH because the positions of the EDTA peaks are sensitive to pH changes

Instrument Start Up - NMR Calibration

- Solution of calibration standard with appropriate bar code is placed on rack and run button pushed
- System checks temperature, lineshape, match and tuning, S/N, pw90, and integral of standard and records the phase
- System shims and adjusts field if needed
- If all parameters are within specifications the tube on the GUI turns green and samples can be run
- Calibration is scheduled for once/24 hours but may be run more often at the labs discretion

Quality Control Monitoring

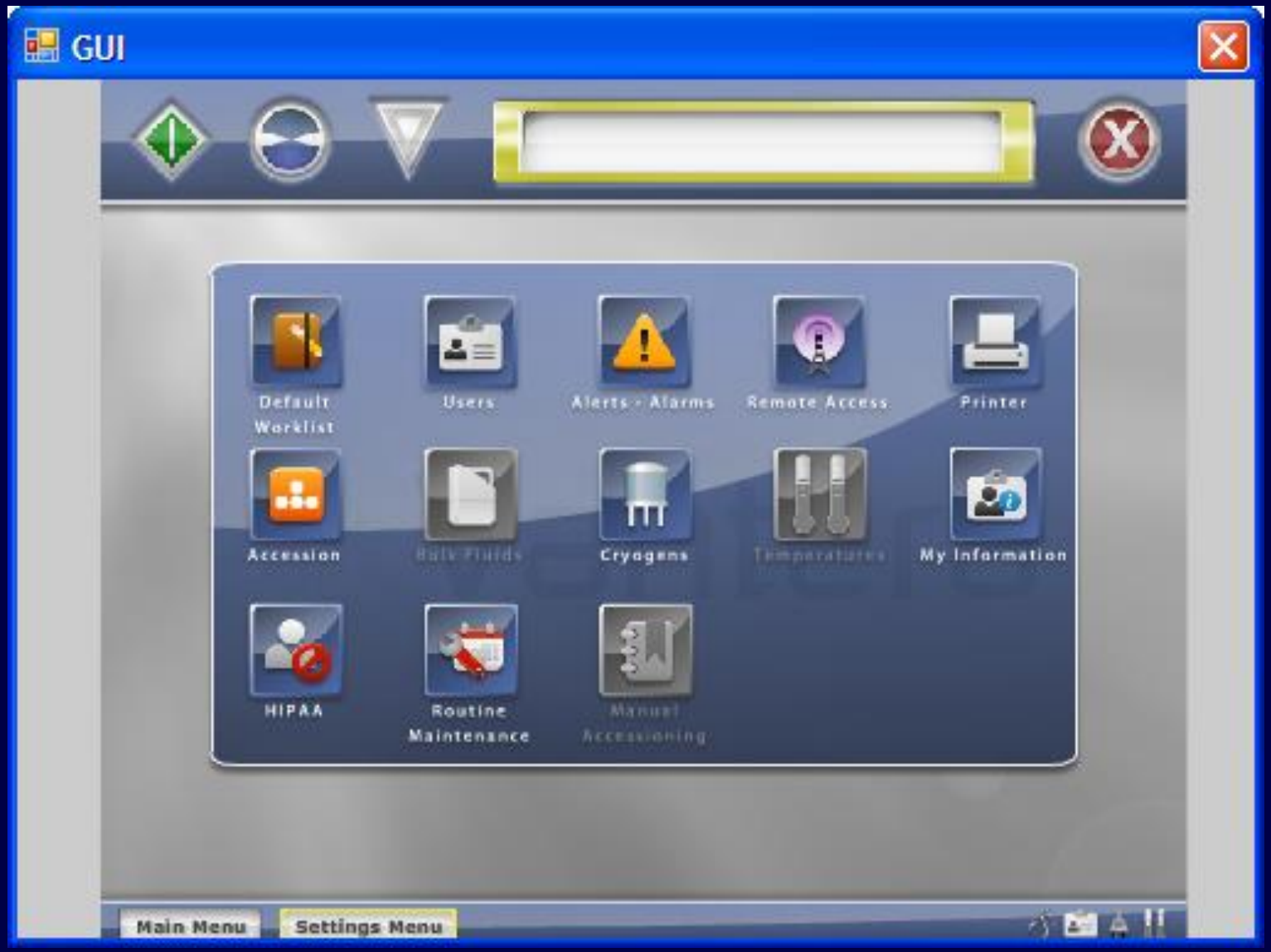
- System temperatures are monitored
- Sample temperature is measured spectroscopically
- Linewidths, concentrations and phases are monitored for each sample
- System will stop and go to “out of calibration” state if it accumulates too many warnings or too many errors

Safety features

- 5 gauss line is within the enclosure
- Moving parts stop when doors are open
- 1-button stop
- UPS with auto-shutdown after 10 minutes with no power

User Interface-Touch Screen

Settings menu



Sample rack in progress

System temperatures

Reagent/Diluent carousel

System Bulk Fluids

Results page

Status of the Technology

- LabCorp purchased LipoScience on Nov. 21, 2014.
- Setup 9 NMR analyzers in their Burlington, NC facility
- Closed Raleigh facility- May 2017
- Have small research group in Research Triangle Park, working on new assay development

Future Analytes

- TMAO - Trimethyl amine N-oxide
- Inflammation Markers
- Branched Chain Amino Acids (BCAA)

Future Instrument Improvements

- Higher magnetic fields
- Smaller magnets
- Smaller electronics
- Gas-cooled probes for higher sensitivity

Team Members

Original Research Group at NC State:

Jim Otvos, Dennis Bennett, Elias Jeyarajah, Irina Shalarova, Qun Zhou

LipoScience:

Mike Peachey
Don Dueul
Matt Clapham
Brad Geddes
Deanna Peaden
Bruce Silberman
Bob Juncosa
Ron Haner
Steve Matyus
Michael Pack
Roger Keim
Claudiu Neagu
Shawn Hurley
Susan Horton