IN VITRO RADIOBIOASSAY

Analytical Methods

Learning Objectives

- Identify analytical methods for alpha, beta and gamma emitters
- Identify considerations for in-vitro bioassay

Radioactivity Measurements

- In order of increasing sample preparation:
  - Gamma-Ray Spectrometry
  - Beta Liquid Scintillation
  - Gross alpha/beta
  - Alpha Spectrometry
  - Radionuclide Specific

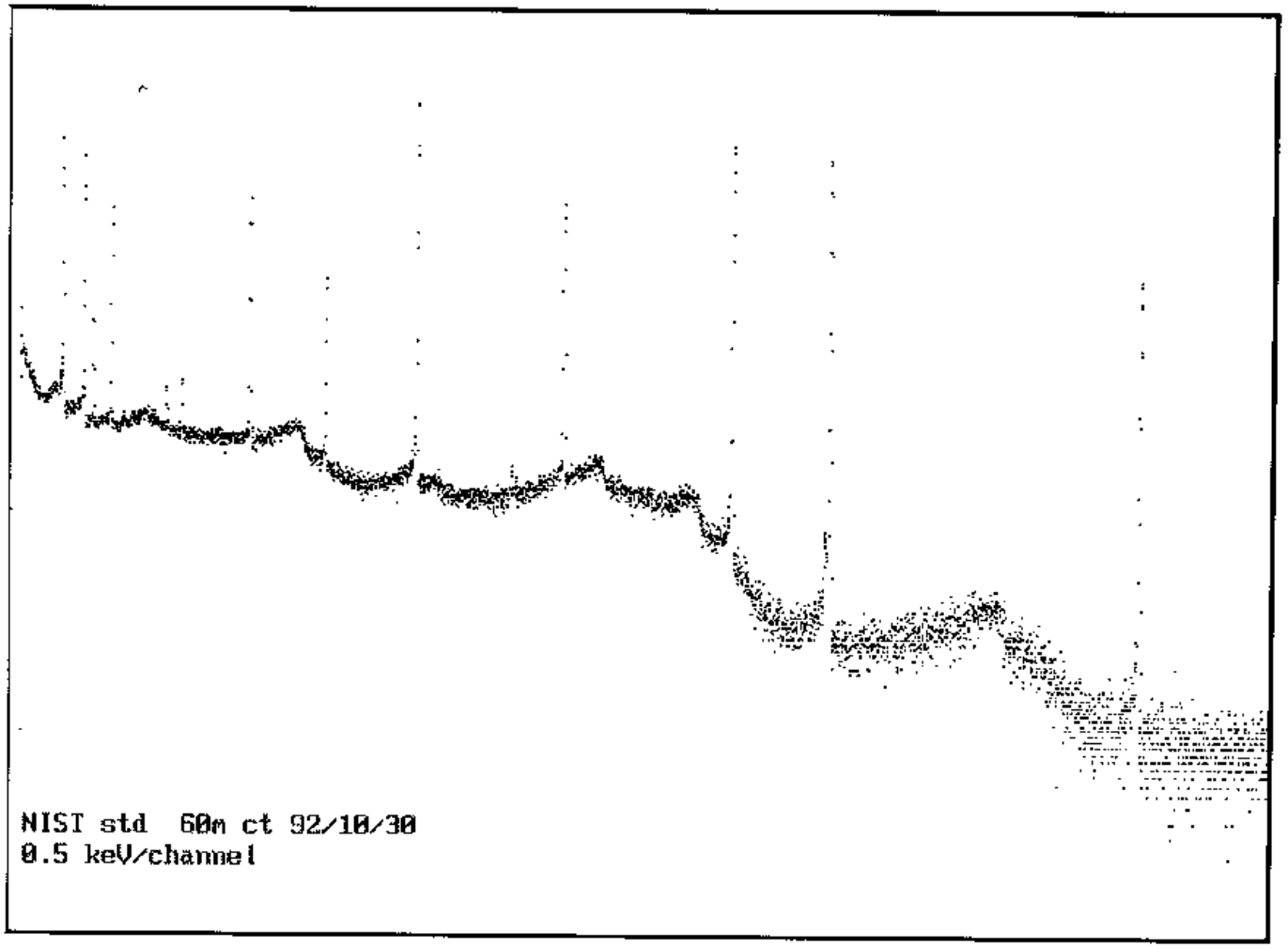
Gamma-Ray Spectrometry

- Minimal sample preparation
- Radionuclide specific
- Low detection limit
- Limited at low photon energy
- Detectors:
  - High-purity germanium (HPGe)
  - Lithium-drifted germanium [Ge(Li)]
  - Sodium lodide [NaI(Tl)]
- Geometries:
  - Marinelli beaker
  - Offset

Gamma Detector Characteristics

- Germanium:
  - small volume and intrinsic efficiency
  - efficiency given as % that of 3”x3” NaI(Tl)
  - high resolution--must for mixed radionuclides
  - operate at liquid nitrogen temperature; Ge(Li) must be kept cold, HPGe may warm up
- NaI(Tl):
  - large volume and intrinsic efficiency
  - lousy resolution--ok for simple spectra
  - generally higher background than Ge (partly because of poor resolution)

HPGe Gamma Spectrum



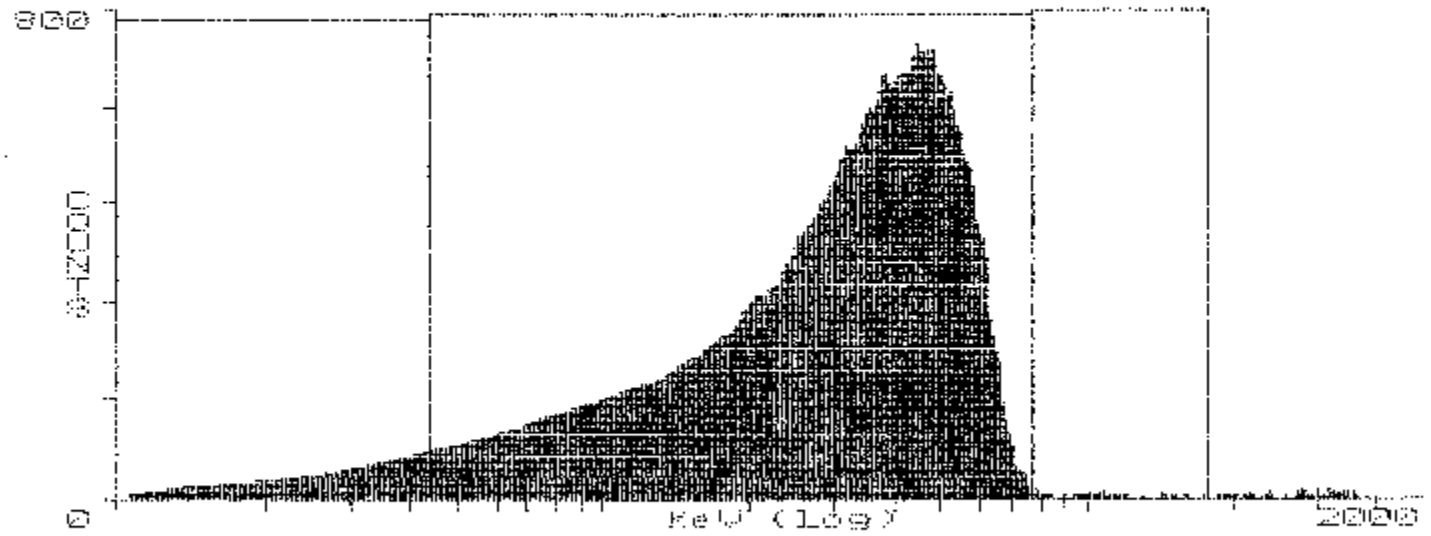
Liquid Scintillation

- Can separate beta emitters into low, medium, and high energy categories
- Newer analytical systems can do alpha-beta discrimination
- Ideal for urine samples--no preparation
- Fecal samples must be dissolved
- Quenching can be a problem
- Background from K-40

Liquid Scintillation (con't)

- Ensure aqueous cocktail is used to avoid creating mixed waste
- Only available method for tritium, plenty of sensitivity
- High throughput--automatic sample changing
- Preferred method for C-14, p-32, and other pure beta emitters

Liquid Scintillation Spectrum



Gross Alpha-Beta

- Sample evaporated to dryness on planchet and counted under gas-filled proportional counter
- No radionuclide identification
- Evaporation causes loss of volatiles
- Not especially sensitive
- Useful only for screening

Alpha Spectrometry

- Usually alpha spectrometric isotope dilution--ASID
- A tracer, or spike is added to sample must ensure isotopic exchange
- Constituents of interest are separated
- A “massless” deposit is obtained--electrodeposition or solvent extraction
- Sample counted with alpha spectrometer

Isotope Dilution

- Need a radioisotope not found in sample
- Must be completely chemically equivalent to analyte of interest
- Usually ensure exchange through aggressive chemical treatment (acid, heating, etc.)
- Activity of unknown analyte =
  activity of spike x $\frac{\text{net analyte counts}}{\text{net spike counts}}$

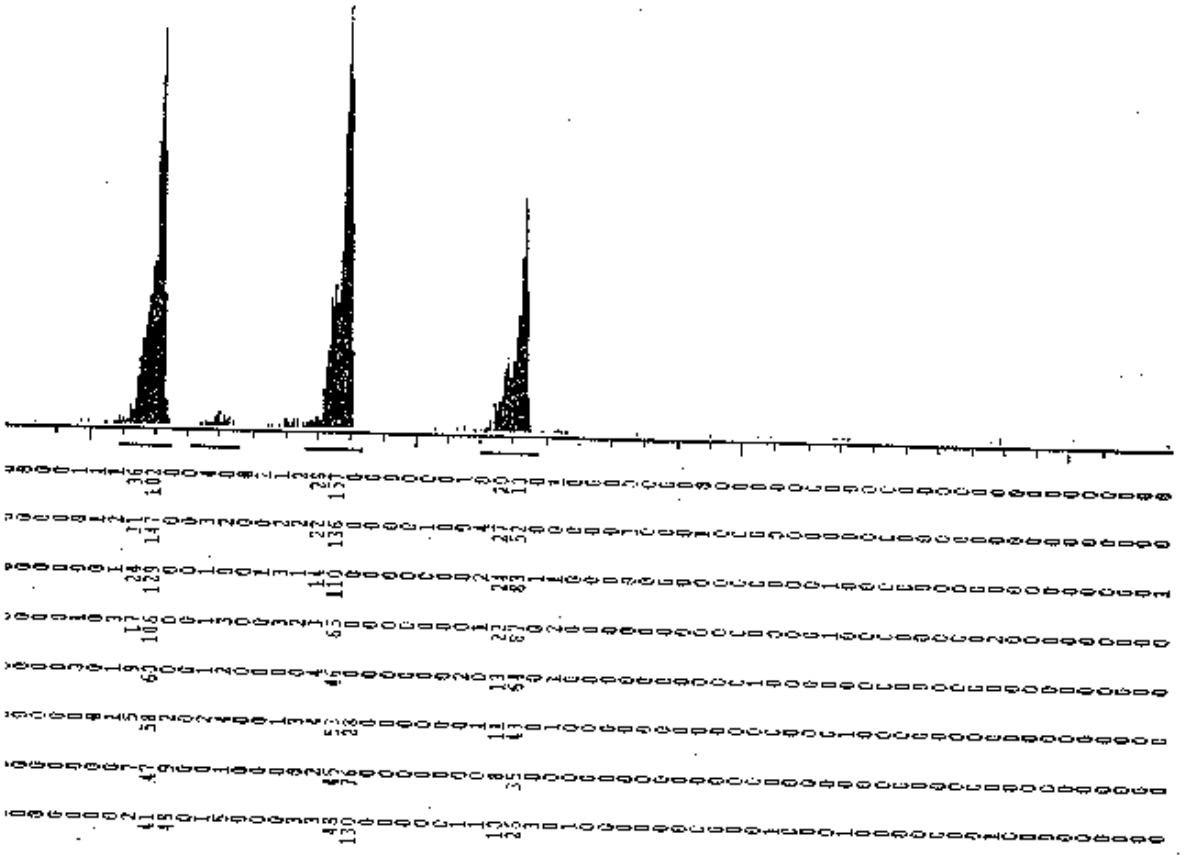
Isotope Dilution (con't)

- Recovery or yield =
  measured spike activity/added spike activity
- Recoveries should be 90% or better; acceptable recoveries should be specified in procedure
- However, can have high recovery without establishing isotopic exchange--need both
- Some examples:
  Pu-236 for Pu-239,240; Am-243 for Am-241;
  U-232 for U-234,235,238
- Spike should not interfere with analyte measurement

Alpha Spectrometers

- Silicon surface barrier detectors
- Efficiency typically >35%
- Very low background:
  0.001 - 0.0001 cpm
- High resolution:
  typically 25 - 30 keV (1σ)
- Need massless deposit to avoid straggling

Alpha Spectrum



Analyte Separation

- Ion-exchange chromatographic resins developed by Horowitz for fuel reprocessing to separate out plutonium and uranium
- Applied to bioassay analysis by Nelson
- Desired analyte is trapped on a column containing resin, while interferences pass through, then eluted
- Resins commercially available, next application is to waste processing

Total Actinides in Urine

- Hot acid digestion (no tracer)
- Coprecipitation with calcium phosphate
- Dissolve in nitric acid
- Adsorb on TRUSpec resin
- Elute with $NH_4HC_2O_4$
- Electrodeposit
- Alpha spectrometry
- Chemical recoveries: U = 89.0+/- 3.6%
- Np = 94.0 +/- 2.4%; Pu = 94.2 +/- 2.2%
- Cm = 95.8 +/- 2.4%; (N = 48 trials)

Actinides in Urine (con't)

- One technician in one day can do 10 samples, 1 blank and 1 spike
- 600 mL urine samples processed
- Overnight counts on Si SBD's
- Detection limit = 0.03 dpm/L of any alpha-emitting actinide
- Cost per sample = $50.

Sensitivity for Pu- 239

- AMDA in ANSI N13.30 =

  0.133 dpm/L

- TRUSpec method has an

  MDA = 0.03 dpm/L and

  L_c = 0.01 dpm/L

- Mean concentration of fallout plutonium in "clean" people =

  0.0008 +/- 0.0032 dpm/L (N = 1251)

Radionuclide Specific Methods

- The radionuclide of interest is chemically separated form the sample and then counted with an appropriate detector-- usually beta
- Do not need to do spectrometry, since only one analyte being counted
- Examples: Sr- 90, Tc-99
- Need to establish average analyte recovery since radioactive spikes usually not used, but chemical spikes (stable Sr) may be

New Techniques

- Pu by fission track analysis
  - Must separate out U-235
  - MDA = 0.001 dpm/L, almost 30 times more sensitive than alpha spec.
  - Cost = $1200 per sample
  - May not be suitable for large-scale routine monitoring
- Pu by mass spectrometry
  - MDA = 0.00002 dpm/L
  - Cost = $ 1000 per sample

More New Techniques

- RIMS
  Resonance Ionization Mass Spectrometry
- SIRIS
  Sputter Initiated Resonance Ionization Spectrometry
- TAMS
  Tandem Accelerator Mass Spectrometry
- RECI
  Resonantly Enhanced Collisional Ionization

Things to Remember

- To run a bioassay lab:
  - You must know what workers are exposed to.
  - Check with your IH people regarding OSHA Lab Hazcom (29CFR1910.1450) and bloodborne pathogens (29CFR1910.1030).
  - Usually don’t need to classify bioassay people as radiation workers.
  - Everything may be subject to review in a court of law someday.