

MATT Meeting May 1, 2008

**An Overview of Special Chemistry in the Clinical
Chemistry Laboratory with Emphasis on the
Measurement of Levetiracetam in Serum/Plasma by
High Performance Liquid Chromatography Utilizing
ChromSystems Kit**

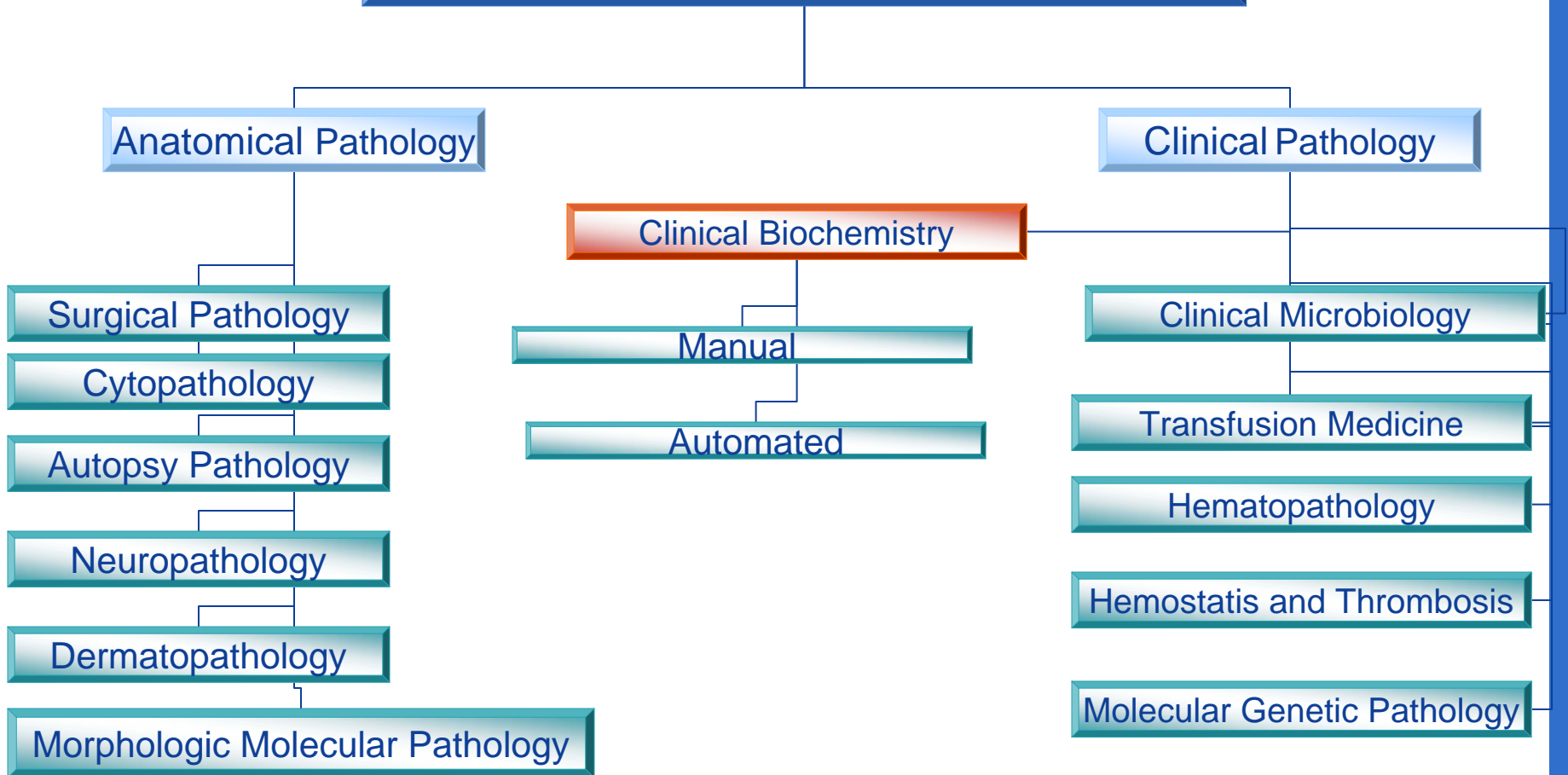
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Pathology and Laboratory Medicine

Pathology and Laboratory Medicine





Annual Volume of Clinical Tests

7.5 Million!

How do we do it???





From Beginning to End

The life of a test tube

1. Collection

2. Transportation

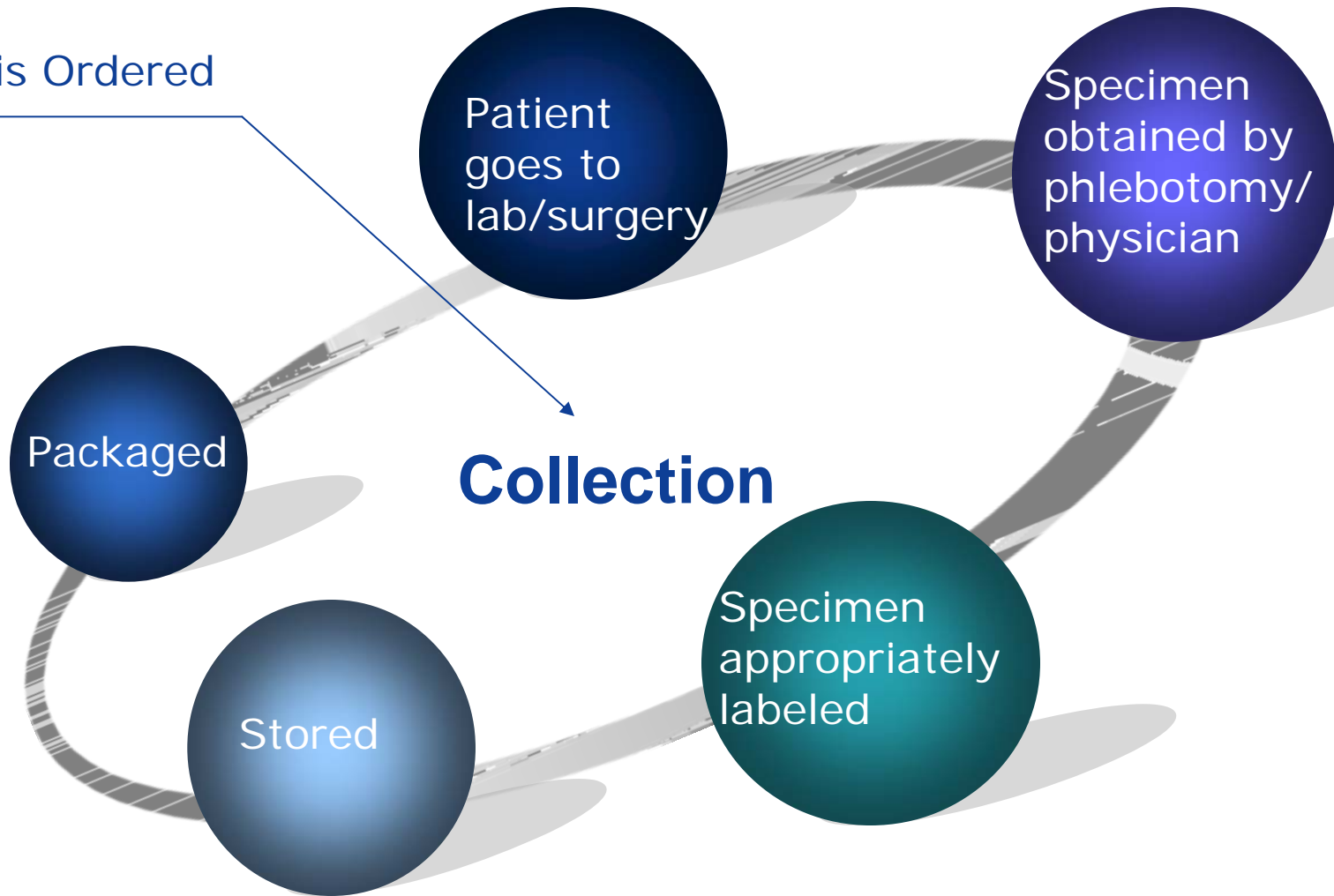
3. Processing

4. Testing and Resulting



Getting the sample

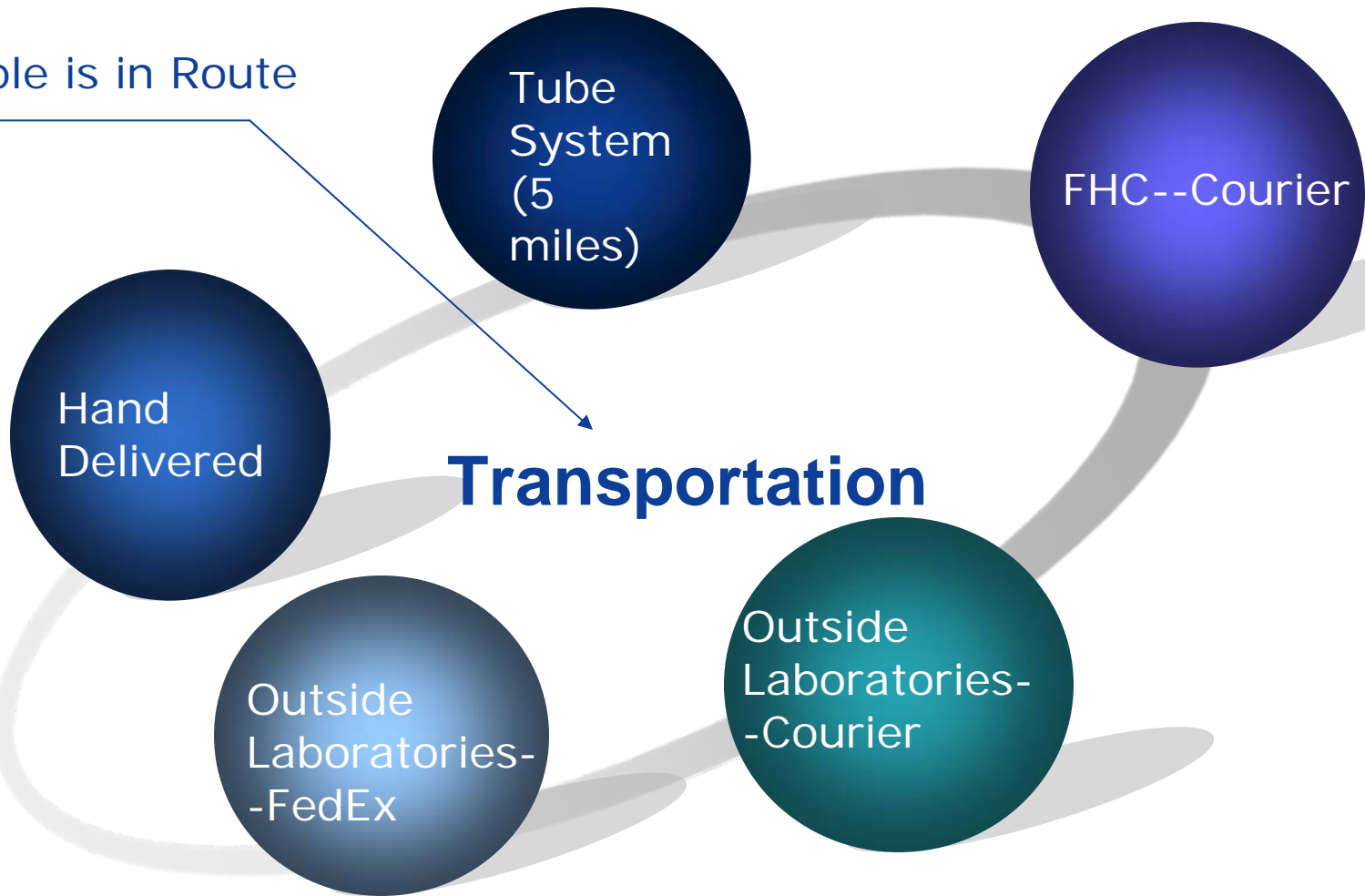
Test is Ordered





Still Getting the sample

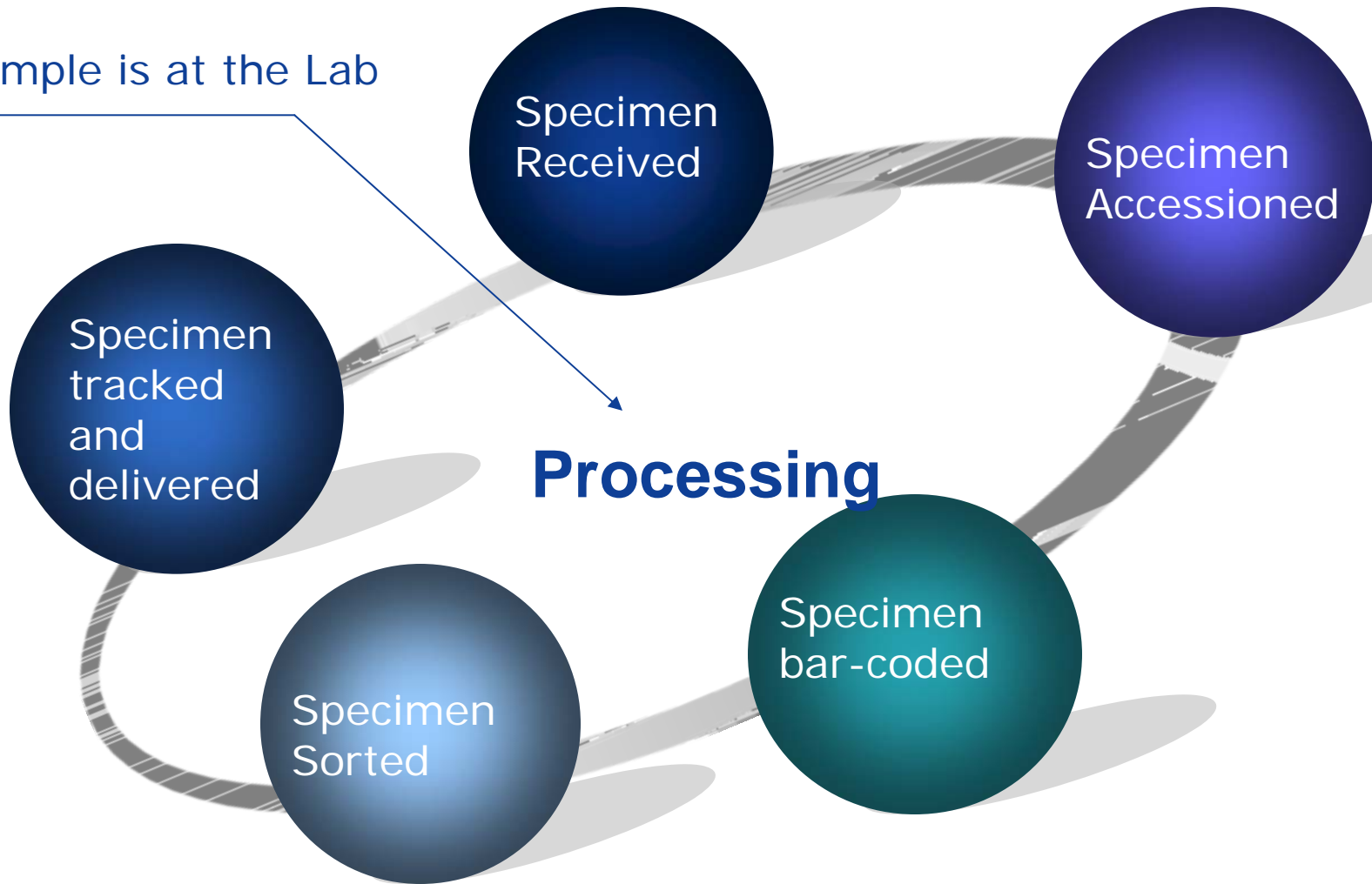
Sample is in Route





Patience—Sample is Coming

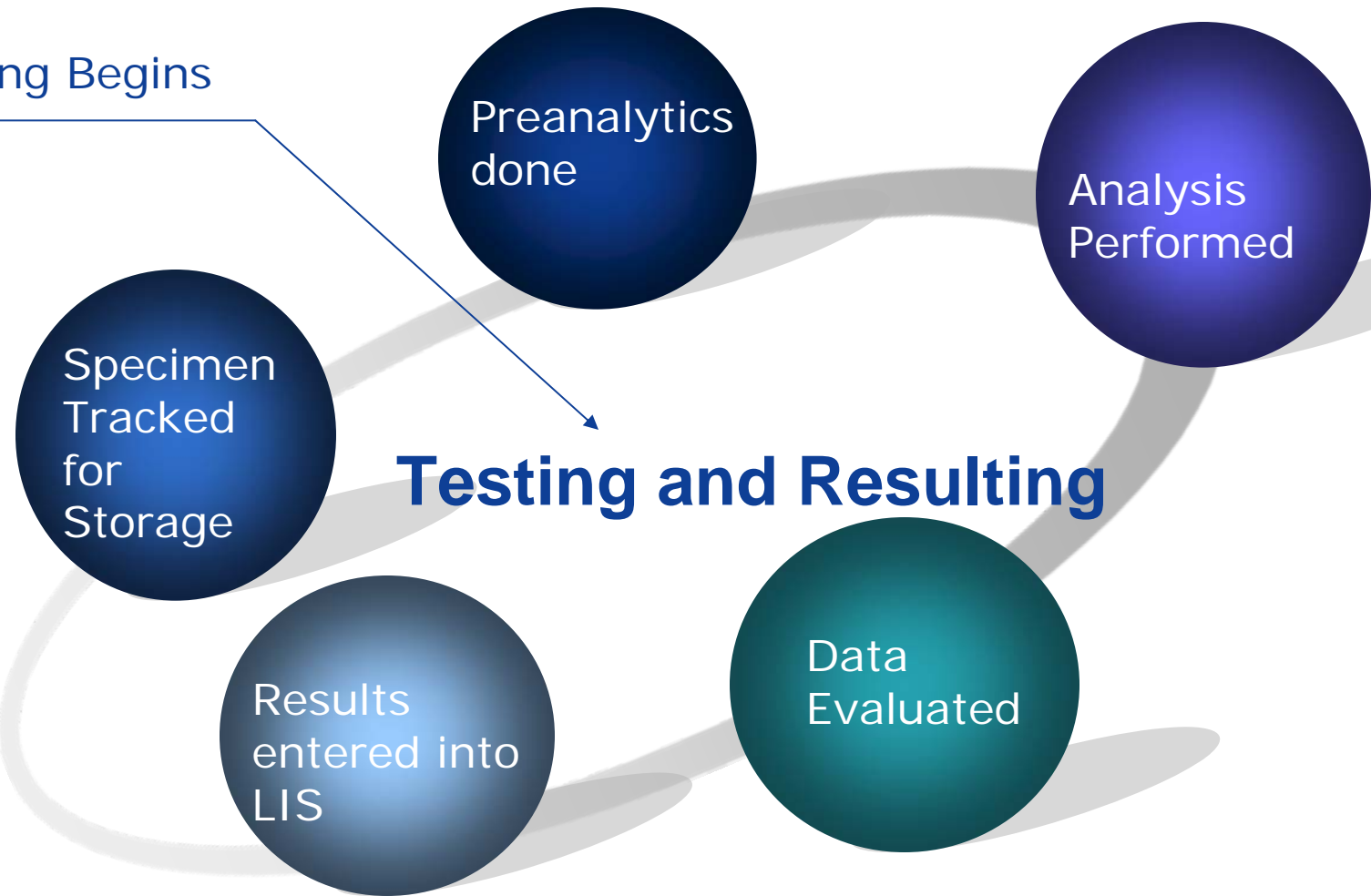
Sample is at the Lab





Sample is in Hand

Testing Begins





Clinical Biochemistry (Manual)

Chromatography/Spectrophotometry (Chrom/Spec)

What Do WE Do?

Toxicology

Therapeutic
Drug
Monitoring

Trace Metals

Kidney
Stone
Analysis

Catecholamines

Lipids
(LS/PG)

Hematology

Miscellaneous



Chrom/Spec Techniques

- HPLC
 - UV
 - ECD
 - FLUORESCENCE
 - MSD
 - MS/MS
- AA
- Immunoassay
- TLC
- GC/MS
- FTIR
- UV/Spec
- Titrations
- Extractions
 - SPE
 - Liquid/Liquid





Evaluating New Drugs

Request

Evaluate

Offer

•Physician
•High Volume
•Interest
Need Improved TAT

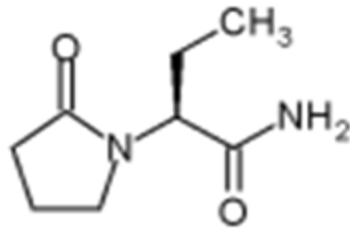
Performance
Characteristics

Test Added to
Directory



What is Levetiracetam (Keppra)?

- Anticonvulsant medication
- Primarily used to treat partial seizure



2-(2-oxopyrrolidin-1-yl)butanamide





Levetiracetam

- Exact mechanism of action is unknown but differs from other anti-epileptic drugs (AEDs)
- Near-perfect pharmacokinetic profile, great tolerance, and no interaction with the metabolism of other AEDs.
- Most commonly reported adverse events are headache, infection, somnolence, and nervousness



Why Monitor Levetiracetam?

- The Doctors want you to!
- The pharmacotoxicological profile is not fully understood. Therefore, monitoring plasma/serum levetiracetam is advisable especially in children and elderly to optimize toxicity and efficacy.





Methodology

- Modified ChromSystems Kit





Reagent Kit Includes

- Mobile Phase
- Calibrators
- Internal Standard
- Solid Phase Extraction Columns (SPE)
- Buffers



Analytical Column

- We used a C8 column
- Can substitute Varian C18 100mg SPE columns for those in Kit



Extraction

- Pipette 100 μ L standard, control or patient in the labeled disposable tubes.
- Pipette 100 μ L Levetiracetam internal standard to each tube. Vortex tubes.

Note for all Vacuum use 1-2 mL/min

- Aspirate 1 mL Equilibration Buffer 1 through each extraction column.
- Aspirate 1 mL Equilibration Buffer 2 through each column.
- Apply and aspirate entire sample\internal standard mixture to the extraction columns.
- Aspirate 1 mL Wash Buffer 1 to each column.
- Aspirate 1 mL Wash Buffer 2 to each column.
- Elute sample with 500 μ L Elution Buffer in a new set of 13x100 glass tubes.
- Mix tubes and transfer the samples to auto sampler vials.



HPLC Parameters

Auto sampler:

Run Time: 7 minutes
Injection volume: 35 μ l

Detector:

Channel: A
Wavelength: 210
AUFS: 1.5
Filter Type: Hamming
Analog Out: Absorbance A
Time Constant: 1.0 sec
Absorbance Offset: 0
Voltage Offset: 0
Chart Polarity: +2
Autozero On: Inject

Pump:

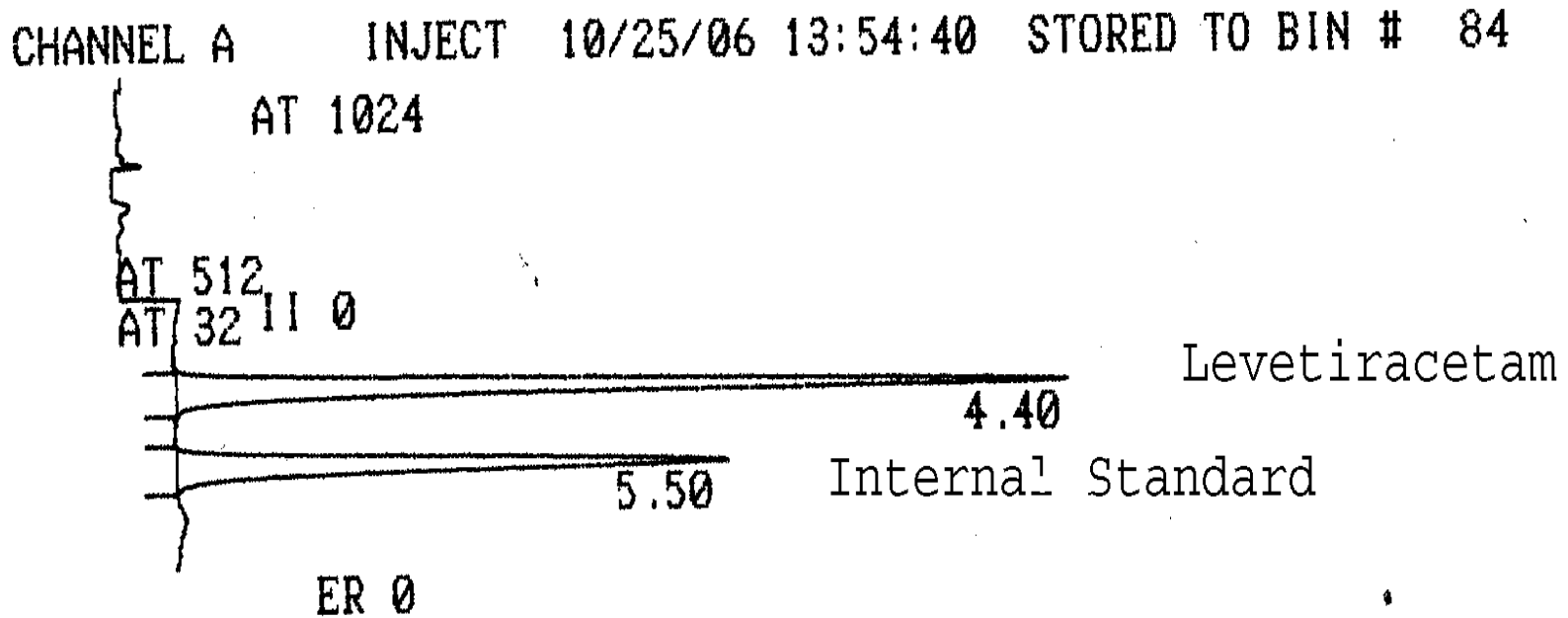
Flow Rate: 1.5 mL/min.
Pressure: <3500 PSI

Integrator:

File (F1) 1
Attenuation (AT) 32
Chart Speed (CS) 0.5
Peak Width (PW) 6
Peak Threshold (PT) 500
Run Time: 7 min



Chromatography



Typical chromatogram of Levetiracetam (22.0 ug/mL) and internal standard.



Method Validation

- Performance Characteristics
 - Limit of Detection
 - Analytical Measurement Range (AMR)
 - Precision
 - Accuracy
 - Recovery
 - Interferences
 - Carry Over
 - Clinically Reportable Range (CRR)



Advantages

- Robust
- Fast
- Cheap
- Easy
- Uses small sample volume



Limit of Detection

The limit of detection was performed by spiking a blank serum sample with a concentration of 1.0 mg/mL of Levetiracetam and running 20 samples.



Analytical Measurement Range

Linearity was performed by diluting an elevated patient with blank serum and assaying in replicate. 1-180 ug/mL

CRR=AMR in this Case

Reference range 5-45 ug/mL



Precision

- **Intra-assay**

- replicates in one batch for two levels of controls

- **Inter-assay precision**

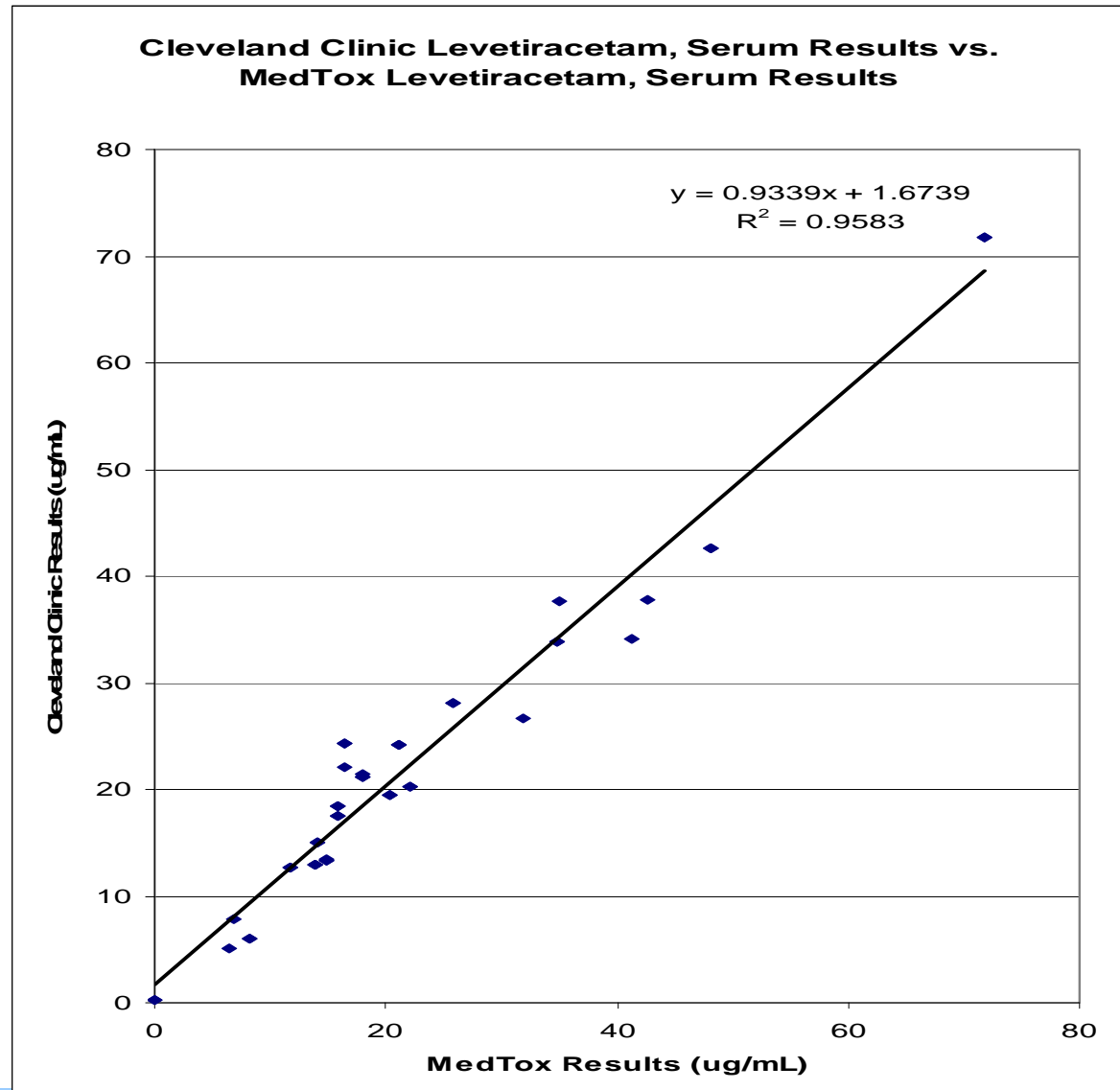
- derived from one run per day for 21 days

	Intra-assay			Inter-assay		
	N	Mean ug/mL	CV	N	Mean ug/mL	CV
Level 1	22	21.8	2.7%	21	8.9	6.0%
Level 2	24	58.9	0.5%	21	53.0	6.1%



Accuracy

Validated through comparison with a commercial GC-MS method (MedTox, Saint Paul, Minnesota) using forty-four de-identified patient .





Recovery

- Analytical recovery was evaluated using a plasma sample spiked with a concentration of 69.8 ug/mL levetiracetam.
- 96.4%



Interferences

Interferences were examined by analyzing multiple commercial controls and spiked serum samples containing therapeutic drugs and endogenous substances. No interferences were found.



Questions

