Clinical Consultation and Results Reporting

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Former affiliation: AIT Laboratories, Indianapolis, IN
The data presented here is based on the work of AIT Laboratories

Outline

- Requisition form
  - Good data collection (patient and medication list)
- Analysis
  - Analytical process
  - Data analysis
  - Automating chromatography data analysis
  - Minimizing the errors
- What should we communicate?
- Approaches to result reporting
Analytical assay

• Urine and Blood
  – Screening - immunoassay
  – Confirmation – mass spec

• Interpretation for urine
  – Positive - detected
  – Negative – not detected
  – Consistent – means consistent with your expectation
  – Inconsistent – means the results inconsistent with your expectation
Urine and Blood

- We screen for the illicit and licit drug on the panel
- Positive results will be confirmed
- Negative results will be confirmed if the medication is listed on the requisition
- Negative results will not be confirmed if the medication is NOT listed on the requisition form
- If the patient on medication, regardless of the negative or positive result we confirm the result.

Analysis (confirmation takes time)

- Analytical procedures
- Data analysis (challenging)
- Reporting

Solution: Automated chromatography data analysis using

AIT Laboratories

- AIT Laboratories Process

AIT Laboratories General Process

2 step review: 2 pairs of eyes, chemist 1 and chemist 2
AIT Laboratories –
Software for data analysis

• Waters MassLynx
• AB Sciex Analyst
• Indigo Biosystems ASCENT

• We use all the three softwares in our laboratories. We use automating chromatographic data analysis for high volume urine tests.

For every 10,000 specimens (x values):
2920 are OPI3 CONF
1764 are BENZO CONF
358 are MTD CONF

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Why did we needed automated data analysis?

• Full Batch Analysis performed by human
  – The “Monday to Friday” difference
  – Variations between shifts (first, second and third shift)
  – Large number of rules to remember
  – Time commitment
automated chromatography data analysis

- Desired Features
  - Easy to use and train staff
  - Consistent
  - Exponentially Modified Gaussian Peak Modeling (EMGP modeling) to identify the peak
  - “QA Rules”

Data analysis

- Instead of “Area Under the Curve,” the software...
  - filters the raw signal using a proprietary algorithm
  - uses the 1st / 2nd derivative to locate possible peaks in the signal
  - uses a “compute engine” to calculate the best Exponentially Modified Gaussian Peak model

Best Exponentially Modified Gaussian Peak Model

Note: Baseline is not straight
Best Exponentially Modified Gaussian Peak Model

Hydrophone

Quant

Quai

15

19/20/13
Baseline

- This is an illustration of correction for a rising baseline.

Data

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QA Rules: for automated data analysis

"QA Rules" are sets of parameters and thresholds that software uses to analyze instrument data. These rules can be simple or complex.

- Chromatograms rules
- QC rules
- Calibration rules
- Concentration/dilution/carryover rules
- Internal standard rules
- etc
QA Rules

- Calibration Concentration Deviation
- Calibration No Intercept
- Calibration Regression Failed
- Calibration Standards Excluded
- Carryover Above LLOQ
- Carryover Flag Subsequent Samples
- Concentration Dilution Required
- Concentration Over Diluted
- Concentration Present but Below LLOQ
- Contamination of Blank Sample
- Internal Standard Area Deviation

Data Correlation Validation: automating the chromatographic data analysis

Temazepam Specimen in

Analyst

Automated integration

Concentration = 797 ng/mL

Concentration = 805 ng/mL
Oxazepam Specimen in
Analyst Automated integration

Concentration = 249 ng/mL
Concentration = 250 ng/mL

Methadone Specimen in
Analyst Automated integration

Concentration = 271 ng/mL
Concentration = 272 ng/mL

Morphine Specimen
Masslynx Automated integration

Demonstration of Peak Resolution (Specimen was reran)
Demonstration of Peak Resolution (Specimen was reran)
**Morphine Specimen in Masslynx Automated integration**

- Concentration = >10000 ng/mL

**Hydromorphone Specimen in Masslynx Automated integration**

- Demonstration of Peak Shape (Specimen is below LLOQ)

**Assay Development**

- **Process steps**
  1. Instrument Data Examination. The data will be used to build the assay in software environment.
  2. “Core QA Rule” adaptation. A list of common rules to the assay are prepared and applied.
  3. Software are installed on each instrument computer.
  4. Finally the assay is refined and is prepared for use. Custom rules can also be implemented.
  5. Validation

- **Estimated time to transfer an assay to automated data analysis** is between 60 and 90 days, depending on complexity and “cleanliness” of the assay.
An allowable deviation of ± 20% (300–450 ng/mL).

Determined concentration of 287 ng/mL.

With the high QC concentration for this analyte being low, any specimen with an Oxymorphone concentration in the range of the LLOQ to the LLOQ (5–50 ng/mL) must be rerun.

This specimen has an oxymorphone concentration of 61 ng/mL and does not need to be rerun.
Flags
Case: High QC low

This specimen has an Oxymorphone concentration of 34 ng/mL, which is in the range for failure (25 – 50 ng/mL). Software informs the user by “flagging” the specimen with the text “Oxymorphone-HQC-LOW.” The reviewer will see the flag and ensure that the specimen is rerun.

Flags
Case: Internal Standard Conc. Low, Over Dilute Specimen

It is flagged, we want the diluted samples to be reviewed. It will tell that it is OD because the value was below LLOQ.

This specimen is flagged for multiple reasons. It’s flagged with “Dilution” simply because the reviewer needs to be aware of that fact. Second, it’s flagged with “OD” because the analyte is Over Dilute. Lastly, the internal standard concentration is below the allowable value, so software is warning the reviewer with the flag "LIS 32.17.”

Benefits of automating chromatographic data analysis.

• Turn-around time
• Consistency
• Access anywhere
• Employee Usage
  – Employees can be analyzing other batches, running or working on instrument, improving processes within the lab
Considerations

- Non-human review in general
- Cost (upfront investment- save later)
- Internet outage (You won’t be able to review your data)
- Data Re-interpretation (anytime instrument data is re-interpreted, you risk changes in peak shapes, concentrations, etc...)
- An issue with one specimen can cause the software to not process an entire batch of specimens.
1. Oxycontin
2. Oxycodone
3. Roxicodone

This is a very popular trick in clinics that rely solely on “instant devices”
This “trick” is very popular in clinics that use only instant devices.

Blood + Urine Analysis

- Urine is a more suitable matrix for determining non-compliant drug use.
- Blood is a more suitable matrix to evaluate the Rx medication due to the additional pharmacokinetic possibilities.
- When collected together, blood AND urine provide the most information possible and aid in identifying:
  - Pill scraping
  - Patients taking infrequent doses
  - Patients dosing shortly before visit
  - Patients over-medicating
  - Slow or non-metabolizers

Blood

- Draw blood to obtain steady-state blood concentrations on high dose opioid patients.
- Only needs to be done once, or every time there is a change in dose.
- Provides pivotal information should the patient die for unforeseen reasons.
### Table 1: Lethal Levels

<table>
<thead>
<tr>
<th>Substance</th>
<th>Lethal Level</th>
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<tbody>
<tr>
<td>Amphetamines</td>
<td>&gt;200 ng/mL</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>&gt;200 ng/mL</td>
</tr>
<tr>
<td>Benzoylecgonidine</td>
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</tr>
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<tr>
<td>Methaqualone</td>
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<tr>
<td>Methocarbamol</td>
<td>&gt;200 ng/mL</td>
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<tr>
<td>Nalorphine</td>
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<tr>
<td>Phenylcyclamine</td>
<td>&gt;200 ng/mL</td>
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<tr>
<td>Picloramide</td>
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<tr>
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<tr>
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### Lethal above 3 ng/mL

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Summary

- Requisition form
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- Analysis
  - Analytical process
  - Data analysis
  - Automating chromatography data analysis
  - Minimizing the errors
- What to communicate in report

Acknowledgment

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