PUTTING IT ALL TOGETHER: MOVING DATA AND REPORTING RESULTS

Judy Stone, Ph.D., MT (ASCP), DABCC
Center for Advanced Laboratory Medicine
University of California, San Diego
jastone@ucsd.edu

Financial disclosure

- Nothing to disclose

Learning objectives

- Define the IT related terms commonly used in a clinical instrument interfacing project
- Explain the advantages of an instrument interface using ASTM/HL7 protocol as compared to a flat file export
- List 4 quality parameters used in LC-MSMS data review that can be automated

Acknowledgements

- Tony DaSilva, Jonathan Agustin, Julia Drees at Kaiser Regional Laboratory
- Shamim Haider, Dave Lavorato, Chris Borton, Hua-fen Liu, John Gibbons at AB Sciex
- Mark George, Don Mason at Waters Corporation
- Don Jarvis at Data Innovations
- David Pope, Melodie Bayongan, John Gaede, Rob Fitzgerald at UC San Diego
- Mike Vogelsberg at SCC Soft Computer

Outline

- How does interfacing your LC-MSMS differ from interfacing an automated chemistry analyzer? *(homework before you start the project)*
- Step by step – project planning to interface your LC-MSMS
- Automating LC-MSMS data review

Acronyms

- Laboratory Information System (LIS)
- Laboratory Information Management System (LIMS)
- **LIS ≠ LIMS**
- Middleware (MW)

The problem

- "Interface" is defined differently by
  1. Clinical chemists, any other laboratorian
  2. Laboratory LIS coordinators and LIS vendors
  3. LC-MSMS vendors

Common LIS Coordinators (Lab) and LIS vendors View

- Interface = connection of a medical device to the LIS for transfer of patient data using standardized protocols that are compliant with health information transfer regulations
- **RS232** connections - instrument to hospital LAN
- **ASTM** (or **HL7**) – standardized data exchange protocol (format & mapping)
- The instrument vendor and the LIS vendor will speak similar jargon, have similar expectations of the task

Jargon Alert: LAN = Local Area Network (Intranet)

Common LC-MSMS Vendors View

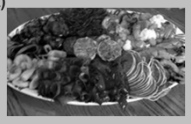
- Interface = export of LC-MSMS data in a **flat file** (generic format) to a shared network directory
- **TCP-IP** connections – instrument to hospital LAN
- .txt or .csv file format with (perhaps) a template customized by the LC-MSMS vendor to control what data fields are exported
- Purchase of an instrument does not automatically include responsibility of the LC-MSMS vendor to customize connectivity and data transfer to the LIS

Jargon Alert: flat file = A flat file is a set of data in a plain text file (.txt) . Each string in the text file holds one record, with fields separated by delimiters, such as commas or tabs.

Jargon Alert: TCP-IP = Transmission Control Protocol/Internet Protocol (*TCP/IP*) is the suite of communi-cations protocols used to connect hosts (computers) on the Internet

Generic

(.txt or .csv file format)



Customizable

Flexible

Handshake

Hand Over

Common laboratorian view

- **"Not in my job description!"**

Explaining the differences - 1

LC-MSMS

- **TCP-IP** connections – instrument to hospital LAN

Automated Chemistry Analyzer

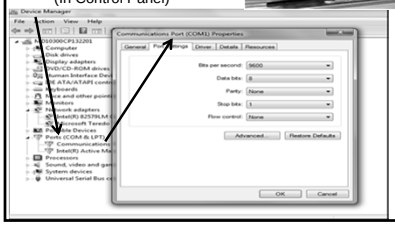
- **RS232** connections - instrument to hospital LAN

Decoding the jargon-Connectivity

Chemistry instrument connectivity

- RS232 - serial or COM port

(In Control Panel)



- **Older**
- **Simpler**
- **Limited in distance from instrument to PC**
- **Must convert to TCP/IP for LAN connection**

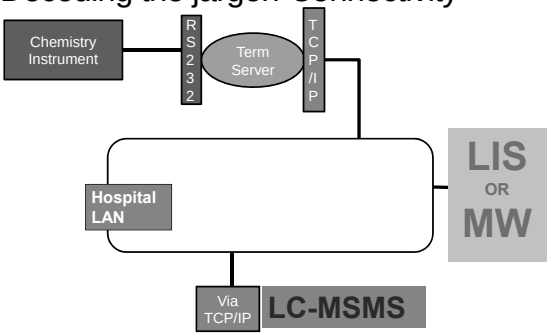
Decoding the jargon-Connectivity

LC-MSMS connectivity

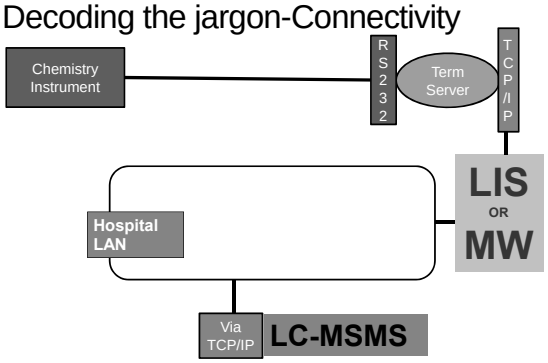
- TCP/IP - network card (NIC) & ethernet cable

- **Newer**
- **Direct connection to LAN, no conversion needed**

Decoding the jargon-Connectivity



Decoding the jargon-Connectivity



Connectivity barriers

- Your Network (LAN) Administrator requires automatic Windows Updates on the PC that controls the MS
- The MS vendor states “No Windows Updates”
- Your Network Administrator requires real time Antivirus scans
- The MS vendor states “Can’t install or run Antivirus”

Work arounds - Antivirus

- Discuss with the MS vendor in advance (include in the RFP) & ask Network Admin. to be flexible about which Antivirus vendor
- Schedule Antivirus updates & PC scanning for times when the instrument is not acquiring
- You can exclude some directories of the MS vendor software from scanning – but that increases risk
- If possible – restrict internet sites on the MS PC

Work arounds – OS Updates

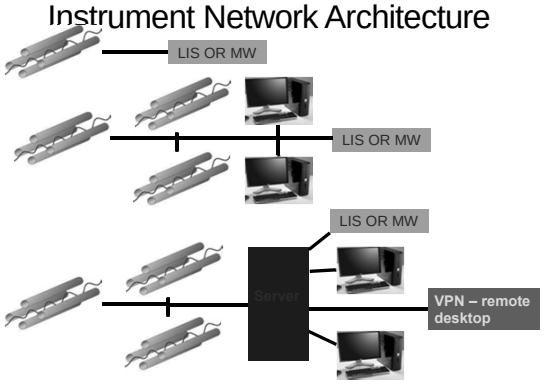
- If a Windows update causes a problem – use Windows Restore Point function to return the PC to it's status quo before updates
- PC configuration - new restore point created before any Windows updates are installed
- Install only "Important", i.e. security related Windows updates
- Configure the PC to download & install, or download with manual install, some time after the 2nd Tues of each month whenever the instrument is not acquiring (e.g., Sunday 5am)

Ease of access versus Security

- Security is a shared responsibility:
  IT, Laboratory, MS vendor
- Include IT in the planning for MS installation
  1. Get agreement on OS Updates & Antivirus
  2. Schedule activation of a Loc Plate near your instrument
  3. Obtain a **static IP address** from Network Admin. or schedule IT to configure for **dynamic IP address (DHCP)**
  4. **Vendor remote access to the MS should be controlled by the laboratory, NOT by the vendor (require "challenged access")**

Jargon Alert: IP Address = PC (network card) unique identifier on the network 10.30.2.181

Instrument Network Architecture



Explaining the differences - 1

LC-MSMS

- **TCP-IP** connections – instrument to hospital LAN

Automated Chemistry Analyzer

- **RS232** connections - instrument to hospital LAN

Explaining the differences - 2

LC-MSMS

- .txt or .csv file format with (perhaps) a template customized by the LC-MSMS vendor to control what data fields are exported

Automated Chemistry Analyzer

- **ASTM** (or **HL7**) – standardized data exchange protocol (format & mapping)

Why do standards & format matter?

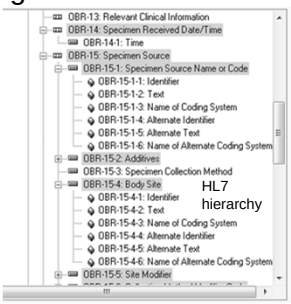
- **Translation** – you call it MRM ratio and I call it 1º Target ratio and she calls it qualifier ratio
- **Location** = the address. Whatever you want to call it – how do I find it in the file?

The same logic for locating houses on a map:

- **Field Name/Tag/HL7 Segment** = apt #, street address, city, state, zip code
- **Field value** = John Doe (who lives there)

Decoding the jargon – Data Exchange Standards

- **ASTM and HL7 are medical data exchange standards for all types of medical information (ASTM came before HL7)**



HL7 hierarchy

D Nelson, Development and Current Use of ASTM/HL7 Messages for Health Care Communications in the USA, Stud Health Technol Inform. 1993;6:138-43.

Good news... but you have to ask:

- The LC-MS vendor may have an interface driver for your LIS/MW **(plug & play)** – ask!
- The LC-MS vendor may have a generic HL7 interface available – ask!
- The LIS vendor may have a robust utility for importing flat files (.txt, .csv) that have been exported from an instrument – ask!
- But be prepared for – flat file export, $$ and time for LIS vendor* to build the interface

Remember – your LIS coordinators and LIS vendor "speak" HL7!

***alternative: In-house programming expertise**

Why a flat file?


```
%PDF-1.3
%âãÏÓ
53 0 obj
<<
/Linearized 1
/O 55
/H [ 2403 456 ]
/L 300652
/E 263086
/N 4
/T 299474
>>
endobj

xref
53 98
0000000016 00000 n
0000002308 00000 n
0000002859 00000 n
0000003326 00000 n
0000003561 00000 n
0000004771 00000 n
0000005000 00000 n
0000005108 00000 n
0000006328 00000 n
0000006350 00000 n
0000007565 00000 n
0000007801 00000 n
0000010091 00000 n
0000010113 00000 n
0000012002 00000 n
0000012024 00000 n
0000012251 00000 n
0000013461 00000 n
```

```
ÐÏà¡±á>þÿ
        ‹'9:;<=>?@
ABCDEFGH-
!"#$%&'()*+,-
./01åæçèéêëìíîïðñ
òóôõö÷øùúûüýþÿ
```


translation you call it mrm ratio and i call it 1º target ratio and she calls it qualifier ratio location the address whatever you want to call it how do I find it in the text string the same logic for locating houses on a map field name tag hl7 segment apt street address city state zip code field value john doe who lives there

Convert formatted & structured to ⟶ generic

.txt file output of an LC-MSMS result table


```
k?xml version="1.0"?>
<QUANDATASET description="" version="1"><XMLFILE filename="C:\Users\Judy\2C
modifiedtime="10:34:51"/><DATASET filename="C:\MassLynxProject\2013\0213.PR
creationdate="04 Feb 2013" creationtime="11:04:18"/><GROUPDATA count="1"><G
\MassLynxProject\2013\0213.PRO\MethDB\Vitamin0_rlf_2_no_smooth.mdb" modifie
creationtime="10:28:20"/><SAMPLELISTDATA filename="C:\MassLynxProject\2013\
count="79"><SAMPLE id="1" groupid="1" name="02012013_1" createdate="01-Feb-
dilutionfac="0.0000000000" extractvolume="0.0000000000" initamount="0.0000C
stdconc="0.0000000000" stockdilutionfac="0.0000000000" subjecttext="" subje
inletmethodname="C:\MassLynxProject\2013\0213.PRO\ACQUDB\Vitamin04" msmethc
postrunmethodname="" switchmethodname="" hplcmethodname="" tunemethodname="
instrument="XEVO-TQS#WAA446" lab="" conditions="" submitter="" task="" user
type="" cas="" stdconc="0.0000000000"><PEAK foundscan="0" foundrt="0.00000C
area="0.0000000000" height="0.0000000000" response="0.0000000000" pkflags="
conccalc="NaN" modifieddate="" modifiedtime="" modifiedtext="" modifieduser
startht="0.0000000000" endht="0.0000000000" absresponse="0.0000000000" rrtr
quanratiowin="0.0000000000" ionratio="0.0000000000" ionratiopred="0.000000C
detectionthreshold="0.0000000000" detectionflag="0" quanthreshold="0.00000C
chromtrace="395.400 &gt; 377.37" peaks="0" pkwidth="0.0000000000" pksigma="
heightdivarea="0.0000000000" baselinewidth="0.0000000000" peakquality="n/a"
recovlimflag="0" matrixblankflag="0" solventblankflag="0" devflag="0" devfl
qcrettimeflag="0" qcpeakshapeflag="0" signoise="" signoiseflag="" cdflag="C
peaktailwidth="0.0000000000" peakasymmetryvalue="0.0000000000" percrecovery
chromnoisehgt="13950.0000000000" concdevperc="0.0000000000" lowerbound1="0.
lowerbound4="0.0000000000" mediumbound1="0.0000000000" mediumbound2="0.000C
upperbound1="0.0000000000" upperbound2="0.0000000000" upperbound3="0.00000C
toxconc1="0.0000000000" toxconc2="0.0000000000" toxconc3="0.0000000000" tox
toxfactor3="0.0000000000" toxfactor4="0.0000000000" toxlod1="0.0000000000"
toxloq1="0.0000000000" toxloq2="0.0000000000" toxloq3="0.0000000000" toxloc
picsreverse="0" iFIT="N/A" iFITnorm="N/A" iFITconfidence="N/A" foundmass="N
iFitconfflag="0" mbaerrorflag="0" ppmerrorflag="0"><CONFIRMATIONIONPEAK1 ic
predrt="0.0000000000" predrrt="0.9894999862" area="0.0000000000" height="0.
empc="0.0000000000" bsanalconc="0.0000000000" conccalc="NaN" modifieddate="
startrt="0.0000000000" endrt="0.0000000000" startht="0.0000000000" endht="C
```


More on file formats

- .txt or .csv — flat file, most generic
- .xml — "markup" language??

XML:
- Data + Format rather than Data only
- Widely used
- Ultimately easier if people and software at both vendors can process XML

- *XML* (Extensible Markup Language) is a flexible way to create common information formats and share both the format and the data on the World Wide Web
- a set of rules for encoding documents in a format that is both human-readable and machine-readable
- is used to define documents with a standard format that can be read by any XML-compatible application. Basically, XML allows you to create a database of information without having an actual database.

Flat file conversion to LIS format

1. Contract with the LIS vendor to do it OR
2. Use in-house programming resources to do it

a.Blank GE, Virji MA. *Development and implementation of an electronic interface for complex clinical laboratory instruments without a vendor-provided data transfer interface.* **J Pathol Inform 2011;2:14**

b. French D, Terrazas E. *The successful implementation of a licensed data management interface between a Sunquest® laboratory information system and an AB SCIEX TM mass spectrometer.* **J Pathol Inform 2013;4:1**

Convert generic to ⟶ **formatted & structured**

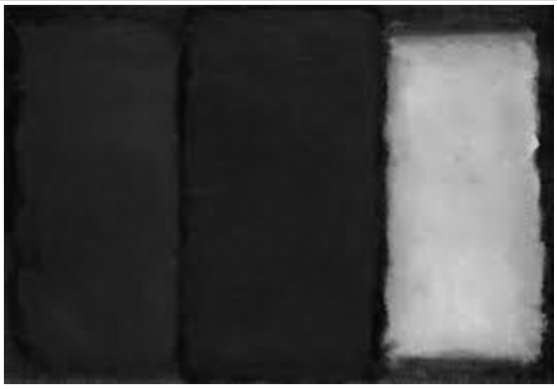
Explaining the differences - 2

LC-MSMS

- .txt or .csv file format with (perhaps) a template customized by the LC-MSMS vendor to control what data fields are exported

Automated Chemistry Analyzer

- **ASTM** (or **HL7**) – standardized data exchange protocol (format & mapping)



Interfacing Workflow A (results upload only)

1. Identify key technical contact people in your LIS and IT groups and at the LC-MS vendor
2. Gather and organize key information, set and communicate goals
   - Your IT group
   - LC-MS vendor
   - Your LIS group & LIS vendor
3. Kick off conference call with all parties (write up and distribute minutes)

Interfacing Workflow B

4. Connect the MS to the network

5. Setup and test connectivity to the LIS/MW

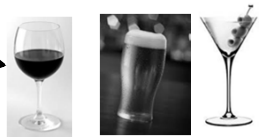
6. Configure/Customize the data that will be exported from the MS
   (select data & format)

7. Mapping (translation tables)

8. Test Transmission in TEST LIS/MW system (send & look at communication traces)

Interfacing Workflow C

9. Troubleshoot & repeat transmission
10. Decision – use LC-MSMS vendor, MW or LIS rules?
11. Test rules, fix rules, retest
12. Test batches, scale up in TEST system
13. Setup in LIVE, repeat all testing
14. Go live
15. Post-live monitoring



Workflow detail

1. Gather and organize key information
   a. IT
      - – what steps are needed to connect to your LAN (OS updates, Antivirus, type of IP address, restricting websites)?
      - Options for instrument network architecture (acquisition to a server)?
   b. LC-MS vendor
      - – what interface options are there (LIS/MW driver, HL7 interface, simple flat file export)?
      - OS updates and antivirus compatibility?
      - Options for data delocalization (stand alone data analysis PCs or acquisition to a server)?
   c. LIS – LIS vendor experience with LC-MS interfacing?

Workflow detail

2. Connecting the MS to the network

a. Two NIC cards in the instrument PC (one to the LC-MSMS and one to the LAN)

b. Live loc plate & a network cable & an IP address

c. TCP/IP connection – not RS232 connection

d. Operating System (OS) i.e. Windows updates

e. Antivirus installation & scanning

Workflow Detail

3. Setup and test connectivity to the LIS/MW

Address for where to send the instrument data?

1. **Simple file export** – shared directory on the network
   - Copy/Paste a file to the shared directory **(file permissions for writing a file to the shared directory?)**
   - Can the LIS/MW capture and read the file?

2. **LIS/MW direct connection –** how to connect?
   - obtain the IP address & port # of LIS/MW
   - Enter them in to the instrument interface configuration screen **(port # is virtual, think TV channel, not a physical plug-in port)**
   - "Ping" the LIS/MW - does it "hear" you?

Workflow Detail

4. How to find the IP address

- ***Click on***  ***type in "cmd"*** 

type "ipconfig /all" (space)

5. How to ping the LIS/MW

- ***Go to CMD prompt ,***
- ***–type "ping ip address"***

(ping 10.10.1.101)

Workflow detail

6. Configure/Customize export content & format from the MSMS

- What fields from your MS data to send (may be >100 fields that can be sent)?
- What are the field names?
- Will you send just the basics or more?

Basics:
Accession #
Test name
Test result
Instrument ID

What is "more"?

Yes or No (Fail or Pass) responses for LC & MSMS quality parameters

- MRM qualifier ratio
- Expected retention time
- Relative retention time
- Internal standard peak area relative to calibrators
- Peak width
- Peak asymmetry
- Calibration characteristics (% deviation, R^2)
- Logical metabolite pattern

Anything you might ask an expert LC-MSMS reviewer to check……

Workflow detail

7. Field Mapping  = round, red thing = tomato

MS field name=LIS field name or HL7 segment

Instrument	HL7	LIS
"Sample ID" =	"**PID-2-1**"=	"Accession Number"
"Serial Number"=	"**OBR-31-2**"=	"Instrument ID"
"Compound"=	"**OBR-31-5**"	= "Test Mnemonic"

Workflow detail

8. Test transmission from MS to LIS/MW – troubleshoot & repeat

- Create test patient orders in the TEST LIS
- Do an LC-MS run with the test patient acc#s
- Review the data with the MS software
- Transmit through the interface to the TEST LIS
- Look at communication trace(s) (if direct to LIS)
- Review in the LIS
- Find the problems and fix them

Simple testing or decide to use rules?

Workflow Detail

9, 10, 11. Setup in LIVE, retest (document), go live!

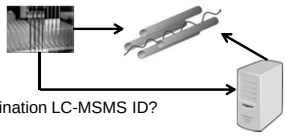
- The myth of "copy to LIVE"
- Any interface build with manual entry (configuration, rules, test names) needs to be tested in LIVE
- Does an LIS TEST system that matches the LIS LIVE system exactly exist anywhere?
- After Go Live - verify some production patients in LIS & EMR LIVE environments

Liquid Handler Interfacing (? Orders Download ?)

- Use automated barcode readers
- Is import (order download from LIS/MW) needed?
- Avoid LIS worklists/batches that require samples to be sorted (by a human)
- Can meta-data from other instruments (e.g., drug immunoassay numeric values) be downloaded to control dilutions/extraction protocols on the liquid handler?

Liquid Handler to MS interface

- Export the plate map from the liquid handler (.txt, .csv, .xls)
  - Well ID (A1, H10)
  - Accession #
  - Plate barcode
  - Test name
  - ? Liquid handler ID/destination LC-MSMS ID?

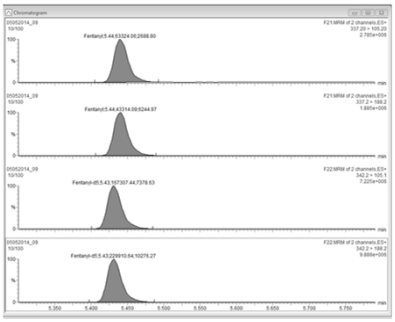


- Export liquid handling errors (clot detection, short sample)?
- Options:
  - import directly to the MS (copy/paste to sample table)
  - OR - Import to the LIS/MW (do LIS/MW drivers exist for your liquid handler?), reformat, and then transfer to the MS

Automating Data Review

- Defining "LC-MSMS Data Review"
- Automating data review & transfer with Rules Engine(s) in the:
  - LC-MSMS software
  - 3rd party software
  - MW
  - LIS

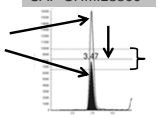
1st - Peak Review



Using LC-MSMS metadata #1

CAP CHM.18800

- **MRM qualifier/quantifier ratio**
  Quantifier MRM (337/105) peak area = 2.8 e6
  Qualifier MRM (337/188) peak area = 1.2 e6
  Qual Peak Area÷Quant Peak Area Ratio = 0.43
  Range = Expected +/- (20% to 30%)

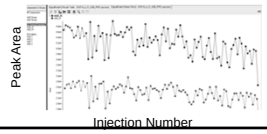


- **Relative Retention Time**
  Retention Time Analyte ÷ Retention Time of Internal Standard
  For example: lorazepam Rt 3.47 min ÷
  lorazepam-d4 Rt 3.45 min = 1.01
  Range = Expected +/- (0.02 min or 3%)

- **Expected = Average of MRM Ratios or RRts found in calibrators from each run**

Using LC-MSMS metadata #2

- Use Internal Standard (I.S.) Peak Areas as an indicator of ion suppression, pipetting errors (no I.S.; twice the I.S.), injection errors (no I.S.)
- Expected I.S. Peak Areas = average of calibrator I.S. Peak Areas in the batch
- Acceptable range = derive from validation data and consensus documents (e.g. +/- 50%)



CAP CHM.18850 and CHM.18900

Using LC-MSMS metadata #3

Additional parameters to consider:

- Abnormal peak shape: Peak Width, Peak Asymmetry
- Calibration errors: R^2, % Deviation (% Accuracy), slope, intercept
- Presence of Metabolites (e.g. EDDP present if Methadone present)
- Any quality parameter you ask an expert LC-MSMS reviewer to evaluate

Setting targets & ranges

- Use "knowns" in each batch or across batches – i.e. calibrators – to set target means or thresholds for each batch
  - Expected RRt or Rt — Rt 2.37 and RRt 1.01
  - Expected Qual MRM Ratio — 43%
  - Expected I.S. Peak Area — 2.34 e5
- Derive acceptable ranges from validation data and consensus standards (e.g. CLSI, WADA)
  - Rt — +/- 0.2 min or +/- 3%; RRt +/- 0.02
  - Qual MRM Ratio — +/- 25%
  - I.S. Peak Area — +/- 50%

Using rules engines – to do what?

1. Identify "quality failures" of LC-MSMS metadata
2. Prevent reporting of results with quality failures (or results outside of the AMR or for gender/age constraints) by:
   a. **Don't export from the LC-MSMS (LC-MSMS vendor software rule)**
   b. **OR don't transfer from MW to LIS (MW rule)**
   c. **OR don't post results to the LIS patient record (LIS rule)**
   d. **OR don't autoverify results in the LIS (LIS rule)**

Rules engines – how to choose?

- Vendor software – minimal to extensive capability, optimal for quality failures, User friendly? User accessible?
- 3rd party generic software – highly flexible, IT resources?
- Middleware – potentially the most robust rules engine & more user accessible than vendor software or LIS but are MS data fields definable?
- LIS – usually least capability
- Commercial 3rd party MS data review automation – most sophisticated, but user accessible? $$?

3rd party SW to ID quality failures

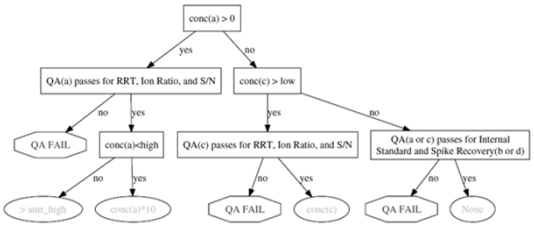
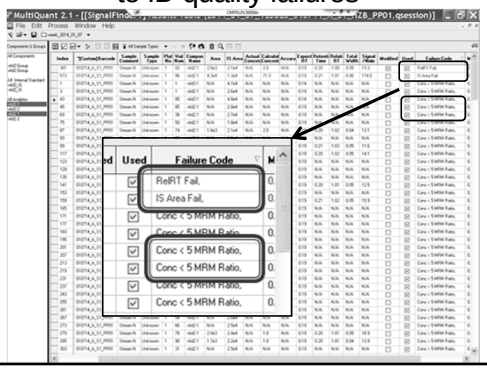


Fig. 4. Flow chart defining the quality control algorithm.

*** Dickerson JA, Schmeling M, Hoofnagle AN, Hoffman NG; Design and implementation of software for automated quality control and data analysis for a complex LC/MS/MS assay for urine opiates and metabolites, Clin Chim Acta , 415 (2013) 290–294.**

LC-MSMS Vendor SW to ID quality failures



MW rules to prevent Tx to LIS

If ⟶ Then

Rules Engine Syntax

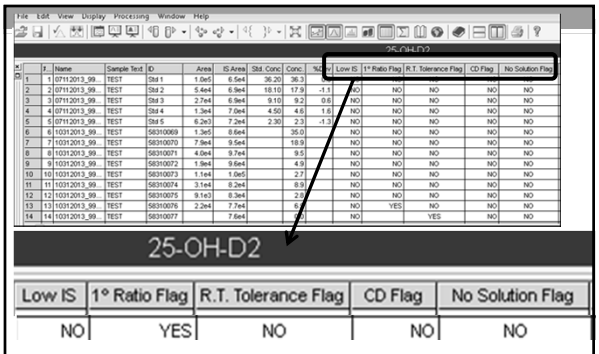
If {Test Result of} "VD2" >"5" AND {"MRMR"} = {"FAIL"} Then {Suppress that test} AND {Print Failure Log}

Description

"If the 25-OH-Vitamin D2 result is >LLOQ and there is an MRM Ratio Failure – then do not post the result in the LIS and print an error log"

Automating Data Review with LIS rules

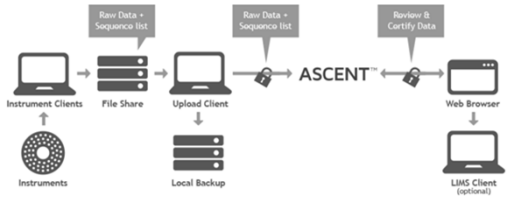
- Waters MassLynx HL7 Interface & SCC SOFT LIS
- Use MassLynx flagging functionality to identify MRM ratio, RRt, Internal Standard Peak area, R^2, No Solution failures
- Send test & flag results to SOFT LIS across HL7 interface (customized data export)
- Write autoverification rules in SOFT LIS. Results with flagging failures inappropriate for concentration don't autoverify.



Entire batch transmitted – problem samples must be suppressed – an individual sample **CANNOT** be removed or excluded from transmission.

Commercial solutions for automating LC-MSMS data review

Indigo Biosystems ASCENT (cloud based)



Workflow detail - #11to #14

Test Rules, Fix Rules & Retest (document)

- Write & follow a testing plan
- First test only **one** rule per result, then test **multiple** rules per result
- After edits – also retest what was working before (**regression** testing)
- Test batches of **genuine patient** samples that include "real life" rule failures

Workflow detail - #11to #14

Test batches, scale up (document)

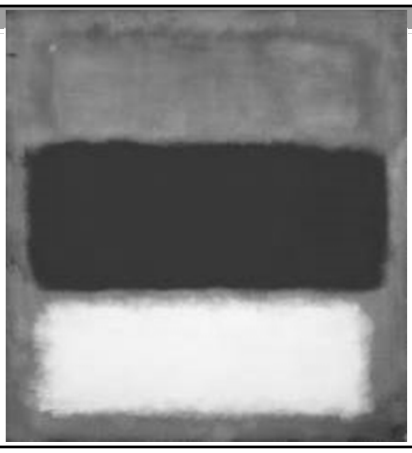
- What works for 1-10 samples may not work for 96 samples
- The best test batch for 2nd tier testing is a typical patient batch with production acc#s replaced by test acc#s

Failures of interface testing -1

1. 5 MS error flags for autoverification rules
2. Tested one error per sample - YES Tested multiple errors per sample - NO
3. "No Peak (NP)" error flag rule – **autoverifies < LLOQ** as the result when no peak is present (no result sent by instrument but NP flag sent as "YES")
4. "Low Internal Standard (I.S.)" error flag rule – **result NOT autoverified** when I.S. is < threshold (I.S. flag sent as "YES")
5. When **both** I.S. and NP flags = YES – result <LLOQ should not autoverify but does because NP flag rule fires first (rule hierarchy). **A sample with no internal standard peak was incorrectly reported as <LLOQ.**

Failures of interface testing - 2

- 10 to 20 samples/batch tested successfully in both TEST and LIVE environments
- Ordering test patients restricted to LIS personnel – no LIS resources to create 96, 192, 288 test orders
- Production starts – every 2-5 batches of 96 fails transmission to middleware (driver code fix needed)



Summary

- Productivity ↑ can also = Error ↑
- Write a testing plan – Test, Test, Test – Document, Document, Document
- No one knows your MS work flow better than you – why not be involved in customizing the automation of that workflow to get exactly what you want?
- You can do it!!