An On-line Tutorial for Bioanalytical Method Validation

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LC-MS/MS has seen enormous growth in clinical laboratories during the last 10-15 years. [1]

- The Neolithic Age(1960-2000):
  - GC-MS
  - Toxicology
- The Bronze Age (past 15 years):
  - LC-MS/MS
  - Small molecules:
    - immunosuppressants (rapamycin)
    - chemotherapeutics (cyclosporins)
  - Multianalyte panels:
    - Steroid Panels
    - Vitamin D
    - Acyl carnitines and amino acids for expanded newborn screening
- The Iron Age (current):
  - Vitamin D and Testosterone
  - Proteins and peptides (Thyroglobulin, IGF-1)
  - MALDI Biotyper : FDA Class II medical device for infectious agent identification

1) https://www.aacc.org/publications/cln/articles/2015/february/future-of-mass-spec; Alan Rockwood/FEB.1.2015 /Clinical Laboratory News

Quest Diagnostics LC-MS/MS Based Assays

- Testosterone (Total and free)
- Aldosterone
- Progesterone
- Estrogens
- Pregnenalone
- Cannabinoids
- Tamoxifen
- 25 (OH) Vit D3
- Thyroglobulin
- Steroids Panel
- 5-HIAA
- HVA
- Serotonin
- Amino Acids
- Metanephrines
- Cortisol
- Carbamazapine
- Etc.

LC-MS/MS assays are almost exclusively laboratory Developed Tests (LDTs)

- LDT is an IVD that is intended for clinical use and designed, manufactured and used within a single laboratory.[2]
- Clinical labs have been using LC-MS almost exclusively for lab-developed tests rather than in combination with FDA-approved assay kits.
- LDTs have so far been regulated by the Centers for Medicare & Medicaid Services under CLIA.
- FDA is now in the process of establishing a framework for regulating the tests as medical devices.[3]
- Public Workshop - Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs), January 8-9, 2015

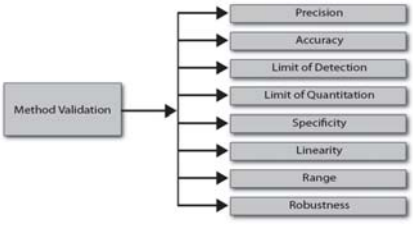
2) 1976 Medical Device Amendments (MDA) of the FD&C Act.
3) http://cen.acs.org/articles/93/i20/Mass-Spec-Welcome-Clinical-Labs.html?h=1756110830

The Regulation is coming but the Guidance is already here:

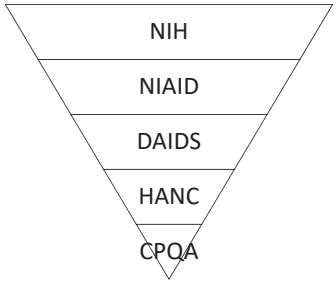
- May 2001/2013: FDA guidance for Bioanalytical Method Validation
  - http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm368107.pdf
- May 2001/2012: CPQA Guidelines for Chromatographic Method Development and Validation based on (and including) FDA Guidelines
  - https://niaid.usalearning.net/pluginfile.php/11701/mod_resource/content/1/CPQA%20Guidelines%20for%20Chromatographic%20Method%20Development%20and%20Validation.pdf
- November 2005: ICH Validation of Analytical Procedures: Text and Methodology
  - https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
- December 2013: LRN CLIA-Compliant Analytical Method Validation Plan and Template
  - https://www.aphl.org/aboutAPHL/publications/Documents/EH_2013Dec_CLIA-Compliant-LRN-C-Method-Validation-Template.pdf
- November 2014: CLSI LC-MS/MS Approved Guidelines
  - http://clsi.org/blog/2014/11/07/clsi-releases-new-clinical-chemistry-document-liquid-chromatography-mass-spectrometry-methods/

Common Validation Parameters

- Accuracy
- Precision
- Selectivity/Specificity
  - Interference
  - Matrix effects
- Sensitivity and detection limits
  - LOD
  - LLOQ
  - ULQ
- Precision
  - Intra-day
  - Inter-day
  - Reproducibility
  - Ruggedness
- Stability
  - Sample
  - Analytical



Bioanalytical method validation in the fight against HIV/AIDS



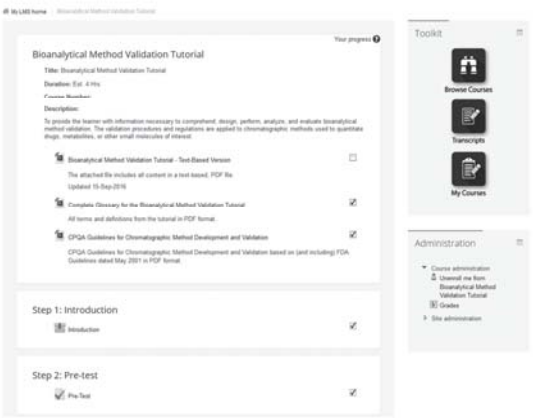
HIV/AIDS Clinical Trials Network and Network Coordination (HANC)

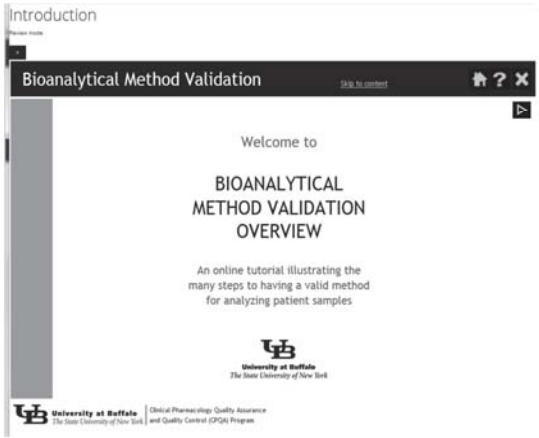
University at Buffalo
The State University of New York

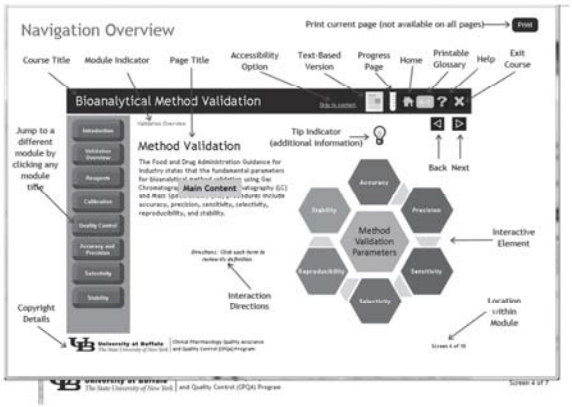
Clinical Pharmacology Quality Assurance and Quality Control Program (CPQA)

- Sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) through DAIDS, 2007 & 2015
- Charged to develop and conduct quality assurance activities for pharmacology laboratories that collaborate in NIAID-sponsored HIV/AIDS networks
- Includes:
  - training and certification for clinical sites and laboratory staff
  - oversight and peer-review of new assay development, validation and implementation
  - proficiency testing program
- Translational Pharmacology Research Core (TPRC)
  - https://tprc.pharm.buffalo.edu/home/tprc/
- Frontier Science & Technology Research Foundation, Inc. (FSTRF)
  - http://www.fstrf.org/
  - Data Coordinating Center for ACTG, IMPAACT, VQA and CPQA
- HIV/AIDS Clinical Pharmacology Quality Assurance and Quality Control (CPQA) Program
  - https://www.fstrf.org/apps/cfmx/apps/cpqa/cpqaDocs/public/index.html

The Bioanalytical Method Validation Tutorial



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Clinical Pharmacology Quality Assurance and Quality Control (CPQA) Program
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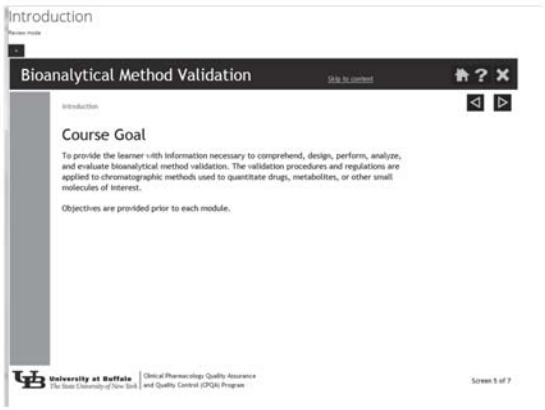
Introduction
Bioanalytical Method Validation
Skip to content
Introduction
Course Goal
To provide the learner with information necessary to comprehend, design, perform, analyze, and evaluate bioanalytical method validation. The validation procedures and regulations are applied to chromatographic methods used to quantitate drugs, metabolites, or other small molecules of interest.
Objectives are provided prior to each module.
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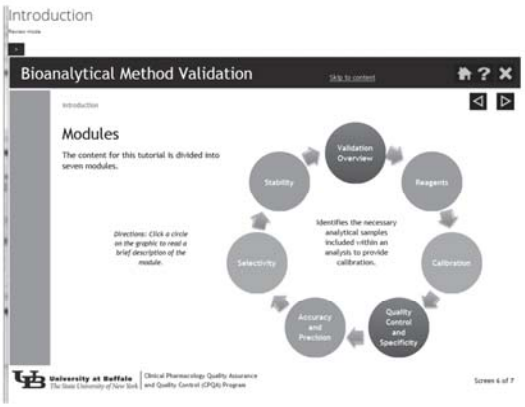
Introduction
Bioanalytical Method Validation
Skip to content
Introduction
Modules
The content for this tutorial is divided into seven modules.
Validation Overview
Reagents
Calibration
Quality Control and Specificity
Accuracy and Precision
Selectivity
Stability
Identifies the necessary analytical samples included within an analysis to provide calibration.
Directions: Click a circle on the graphic to read a brief description of the module.
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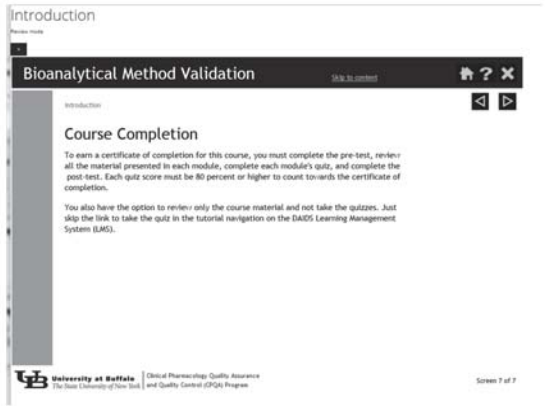
Screen 6 of 7


Introduction
Bioanalytical Method Validation
Skip to content
Introduction
Course Completion
To earn a certificate of completion for this course, you must complete the pre-test, review all the material presented in each module, complete each module's quiz, and complete the post-test. Each quiz score must be 80 percent or higher to count towards the certificate of completion.
You also have the option to review only the course material and not take the quizzes. Just skip the link to take the quiz in the tutorial navigation on the DAIDS Learning Management System (LMS).
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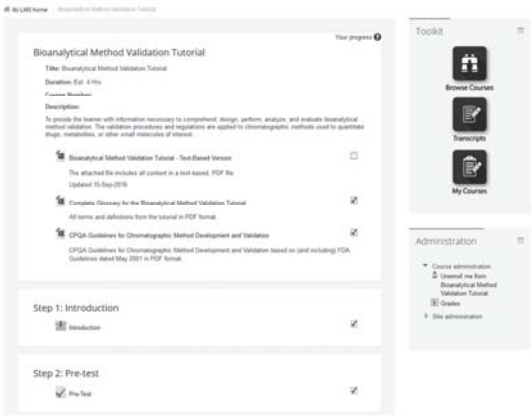
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Step 1: Introduction
Step 2: Pre-test


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USA.gov

Step 3: Validation Overview

Read content; pass quiz with score of 80% or higher

- Validation Overview presentation
- Validation Overview Quiz
- Clinical Laboratory Improvement Amendments Brochure

  A brochure about the Clinical Laboratory Improvements Amendments (CLIA) from the Department of Health and Human Services in PDF format.
- Guidance for Industry - Bioanalytical Method Validation, May 2001

  Guidance for Industry on Bioanalytical Method Validation from the U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine in PDF format.
- Statistical Parameter Definitions and Calculations

  This document is a reference to calculating mean, standard deviation, %Coefficient of Variation, and %Deviation as used throughout the tutorial.

Step 4: Reagents

Read content; pass quiz with score of 80% or higher

- Reagents - presentation
- Reagents Quiz
- CPQA Certificate of Authenticity Example

  Sample Certificate of Authenticity (COA) from the CPQA.

Step 5: Calibration

Read content; pass quiz with score of 80% or higher

- Calibration - presentation
- Calibration Quiz

Step 6: Quality Control

Read content; pass quiz with score of 80% or higher

- Quality Control - presentation
- Quality Control Quiz

Step 7: Accuracy and Precision

Read content; pass quiz with score of 80% or higher

- Accuracy and Precision - presentation
- Accuracy and Precision Quiz

Step 8: Selectivity

Read content; pass quiz with score of 80% or higher

- Selectivity - presentation
- Selectivity Quiz
- Testing for Matrix Effects Mass Spectrometry Reference Sheets

  This reference sheet contains a copy of the data presented in the Selectivity Module in PDF format.

Calibration - presentation

Bioanalytical Method Validation

Skip to content

Calibration

Calibration

Calibration describes the relationship between analytical instrument response and theoretical concentration. The FDA requires a minimum of six calibration standards be included in each analysis performed.

Directions: Click the test tube to the right to access the Calibration Activity.

Attention Google Chrome users: Please hold down the Shift key when clicking this icon. This will ensure it appears in a new window rather than on a new tab.

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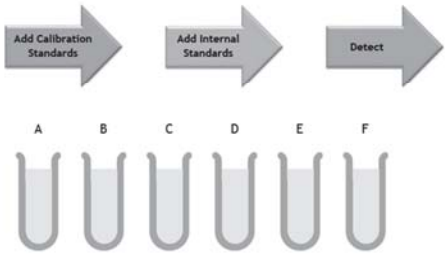
Calibration Standards Preparation

Print

Six tubes appear below to prepare calibration standards of 100, 200, 300, 400, 500, and 600 ng/mL matrix. Click each arrow from left to right to prepare, process, and detect the calibration standards.



Calibration Standards Preparation

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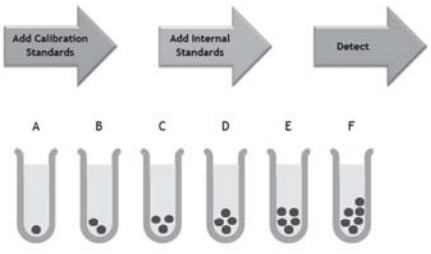
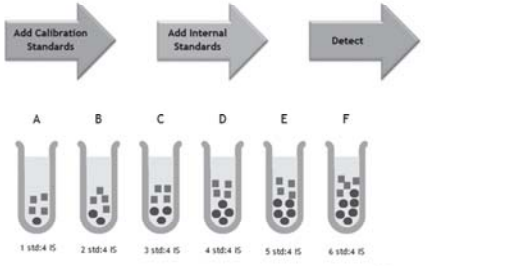


● = Calibration Standard (std)

Calibration Standards Preparation

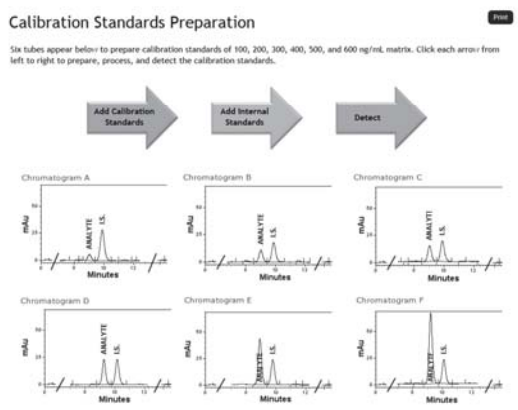
Print

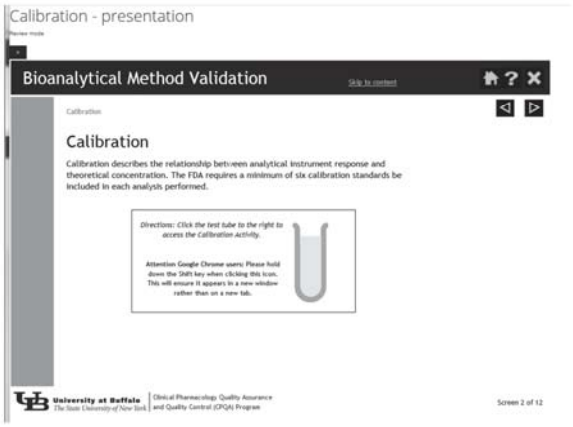
Six tubes appear below to prepare calibration standards of 100, 200, 300, 400, 500, and 600 ng/mL matrix. Click each arrow from left to right to prepare, process, and detect the calibration standards.

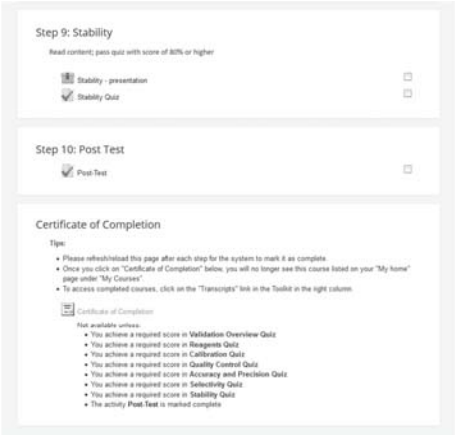


● = Calibration Standard (std)

■ = Internal Standard (IS)







Summary Outcomes (2/2/2017)

	Count						
Started*	57						
Completed	29						
	%						
Avg. Pre-test	63.13						
Avg. Post-test	85.19						
Educational Level	**2-yr**	**4-yr**	**MS**	**PhD**	**MD**	**Other**	
% of completed	7	62	25	4	0	2	
Network	**ACTG**	**HPTN**	**IMPAACT**	**INSIGHT**	**MTN**	**VTN**	**Educational**
% of Completed	24	21	4	4	10	10	49
Evaluation (%)	**Overall**	**Utility**	**Organization**	**Level**			
Very Satisfied	39	56	28	45			
Satisfied	61	39	72	50			
Unsatisfied	0	0	0	0			
No Opinion	0	5	0	5			

* Includes demonstrations and trials

Accessing the Bioanalytical Method Validation Course on the DAIDS LMS

Go to the DAIDS Learning Portal (http://www.daidslearningportal.com).

Request an account if you do not have one. Click Go to LMS when you have your credentials.

Registering your students

DAIDS Learning Portal — Multiple Account Request Form

Please send the completed form to the DAIDS Training Support Team at daidscrss-support@westat.com.

Name of SITE:	Your Institution
SITE ID:	Your DAIDS LMS Account #
Contact:	Your Name

	First Name (*required)	Last Name (*required)	E-mail Address (*required)	Role (*required)
1				
2				
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The BMV Team

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Distinguished Professor
Director of the Translational Pharmacology Research Core
CPQA Principle Investigator