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Clinical Pathology Peer Review

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Summary

- Anatomic Pathology Peer Review (Formal , Documented)
  - Qualitative, subjectively generated data
  - Peer review is primarily driven by a need to improve confidence in the accuracy of the data
- Routine review of Clinical Pathology by study scientists is sufficient
  - QC/QA: Most Clinical Pathology data is objective, quantitative and validated for accuracy
  - Informal review by multiple experts enhances data integration and consensus
- Clinical Pathology Peer Review is rarely needed, but if performed:
  - Should be conducted by a Toxicologic/Industry Clinical Pathologist
  - Formal Clinical Pathology Peer Review may be useful in rare instances

Tomlinson, L. et al., 2013. Best practices for veterinary toxicologic clinical pathology, with emphasis on the pharmaceutical and biotechnology industries. *Veterinary Clinical Pathology*, 42(3), pp.252–269.

SUMMARY

- Considered separate from APPR, SD or Management review
- Should be performed by a qualified individual (Toxicologic/Industry Veterinary Clinical Pathologist)
  - Must have the training and experience to competently evaluate all clinical pathology data within the context of the entire study (eg. Toxicologic/Industry Veterinary Clinical Pathologist).
  - Should be experienced with studies using the same analytes/biomarkers, species and of similar duration and design as the study to be peer reviewed.
  - Contributions of individuals with other specialized expertise engaged to consult on specific topics should be limited to their areas of expertise.

Adapted from: Morton, D. et al., 2010. Recommendations for pathology peer review. *Toxicologic Pathology*, 38(7), pp.1118–1127.

Compare and Contrast

Clinical Pathology	Anatomic Pathology
Machines and Numbers	Blood and Guts
Instruments, test tubes and microscopes	Microscopes and naked eye
Molecules, Cells, Body Fluids, Homeostasis, Internal Medicine, Clinical Medicine, Clinical Condition	Cells, Extracellular Matrix, Organs, Tissues, Architecture, Necropsy, Pathophysiological process
Quantitative data generated by an instrument	**Qualitative** data generated by visual examination
Reflects systemic changes	Reflects local changes (section evaluated may not contain the lesion)
Reflects functional/biochemical and structural alterations/injury	Reflects structural injuries/alterations
Non-invasive: Useful to monitor changes over time in animals and in the clinic	Invasive: Requires necropsy/autopsy or biopsy
Generally High sensitivity: able to detect subtle changes	High Specificity: Provides a high level of certainty regarding presence of lesions

Anatomic Pathology PR

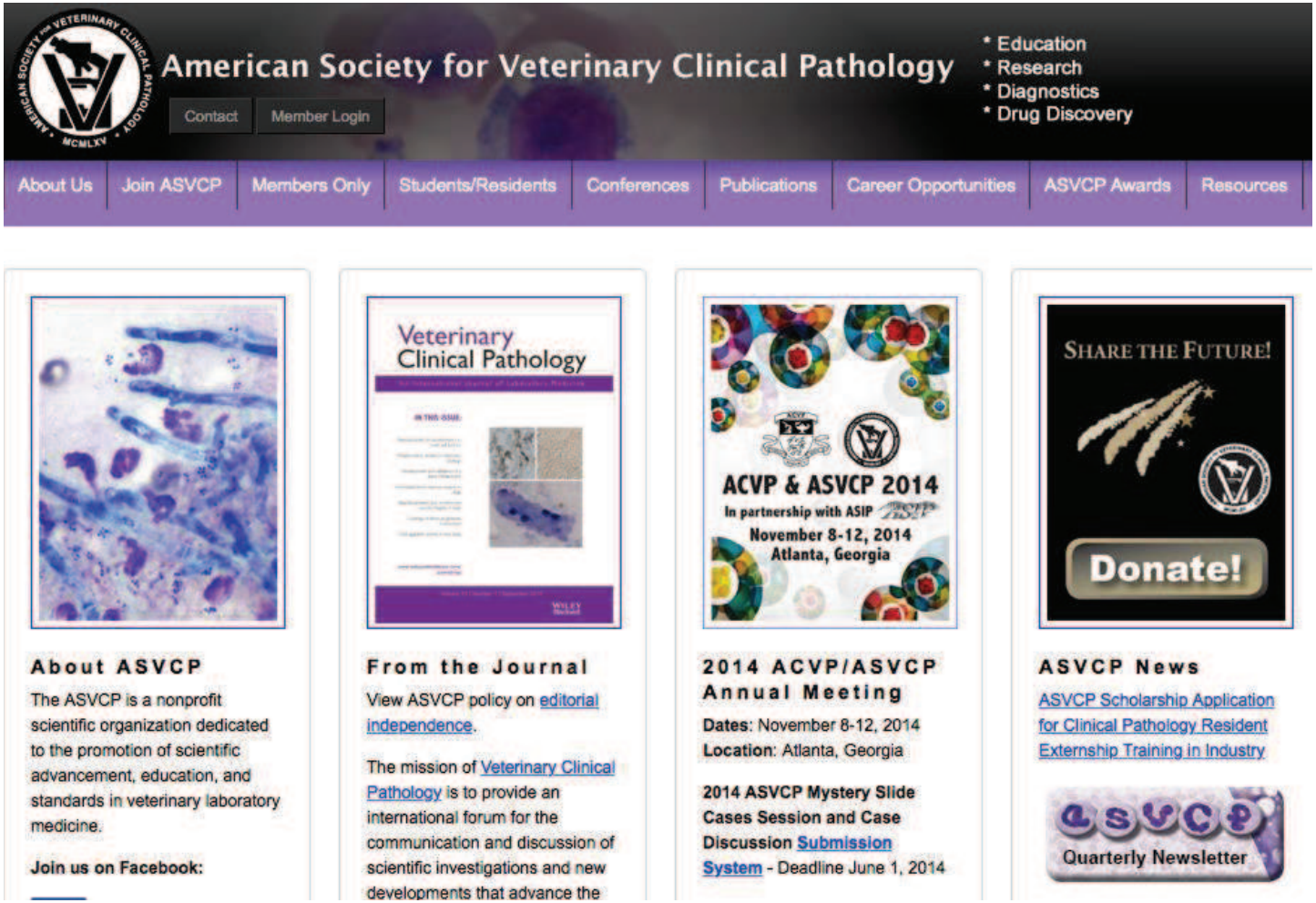
- Histopathology data
  - Qualitative and subjective by nature
  - Often viewed by quality assurance professions and other non-pathologists using the data with a certain degree of skepticism
- The objectives of a histopathology peer review:
  - Increase confidence in the **accuracy** of histopathology findings
  - Ensure **consistency of nomenclature**
  - Confirm **completeness** (undiagnosed TA-related lesions)
  - Review the **correctness of the textual interpretations**
  - Determine the **appropriateness of the NOEL or NOAEL** *(should be a team effort with the Study director and Clinical Pathologist)*

Crissman, J.W., Goodman, D.G. & Hildebrandt, P.K., 2004. Society of Toxicologic Pathology Guideline. … *Pathology*.

Confidence In Clinical Pathology Data

- Data is mostly quantitative and obtained using objective/automated, validated methods
- Data is under the scrutiny of SOP-driven quality control processes to ensure accuracy
- Ensured by a Quality Control process and Validation of Laboratory Information Management Systems
- Documented via GLP compliance mechanisms (Deviations)

American Society for Veterinary Clinical Pathology (ASVCP)



ASVCP Quality Assurance and Standard Guidelines

Point-of-Care Testing Guideline

Flatland, B. et al., 2013. ASVCP guidelines: quality assurance for point-of-care testing in veterinary medicine. *Veterinary Clinical Pathology*, 42(4), pp.405–423.

Reference Interval Guideline

Friedrichs, K.R. et al., 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology*, 41(4), pp.441–453.

Allowable Total Error for Biochemistry

Harr, K.E. et al., 2013. ASVCP guidelines: allowable total error guidelines for biochemistry. *Veterinary Clinical Pathology*, 42(4), pp.424–436.

General Quality Assurance Guidelines

Flatland, B. et al., 2010. ASVCP quality assurance guidelines: **control of general analytical factors** in veterinary laboratories. *Veterinary Clinical Pathology*, 39(3), pp.264–277.

Vap, L.M. et al., 2012. ASVCP quality assurance guidelines: control of preanalytical and analytical factors for **hematology** for mammalian and nonmammalian species, hemostasis, and crossmatching in veterinary laboratories. *Veterinary Clinical Pathology*, 41(1), pp.8–17.

Gunn-Christie, R.G. et al., 2012. ASVCP quality assurance guidelines: control of preanalytical, analytical, and postanalytical factors for **urinalysis, cytology, and clinical chemistry** in veterinary laboratories. *Veterinary Clinical Pathology*, 41(1), pp.18–26.

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SPECIAL REPORT

Best practices for veterinary toxicologic clinical pathology, with emphasis on the pharmaceutical and biotechnology industries

Lindsay Tomlinson[1], Laura I. Boone[2], Lila Ramaiah[3], Kelley A. Penraat[4], Barbara R. von Beust[5], Mehrdad Ameri[6], Florence M. Poitout-Belissent[7], Kurt Weingand[8], Heather C. Workman[2], Adam D. Aulbach[9], Dennis J. Meyer[7], Diane E. Brown[10], Amy L. MacNeill[11], Anne Provencher Bolliger[7], Denise I. Bounous[12]

- Good laboratory practices (GLP)
- Laboratory informatics and management systems (LIMS), quality control (QC), and quality assurance (QA) programs
- Instrument and Method Validation

Tomlinson, L. et al., 2013. Best practices for veterinary toxicologic clinical pathology, with emphasis on the pharmaceutical and biotechnology industries. *Veterinary Clinical Pathology*, 42(3), pp.252–269.

Good laboratory practices (GLP) and Quality Assurance (QA)

- Laboratories testing nonclinical samples in support of FDA/ EPA/OECD applications are required to comply with GLP
  - **Standard practices** for validation, certification, and/or qualification of all operations including personnel, facilities, systems, **equipment, methods, reagents, sampling, sample handling, sample testing, stability testing, and raw data management**).
  - Require **documentation**, review and approval of test results, data recording and reporting, as well as plans for corrective action, risk management, and disqualification of testing facilities.
  - Compliance directed by the use of SOPs.
- QA refers to audit or verification processes performed by an independent QA group that ensures compliance with GLP regulations
- QA validation verifies that laboratory procedures specifically comply with GLP regulations

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Laboratory informatics and management systems (LIMS)

- Systems for the identification, assignment, collection, management, transfer, analysis and storage of electronic data (results and QC) and records should be validated.
- Audit trail for all instrument-related activities: date and time of analysis, identity of the instrument, and personnel who ran the samples and signed as responsible for the raw data.
- Documentation of any change to an instrument's original set up or modifications/editing of data, with time/day of change, and reason for the change (eg. outliers due to preanalytical/analytical parameters).

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Quality Control (QC)

- QC generally ensures consistent practices in the laboratory associated with generating and reporting quality data.
- Internal QC:
  - Consists of planned and systematic monitoring of facilities, equipment, personnel, methods, practices, records, and controls
  - Ensures that the lab conforms to applicable regulations
  - Verifies that data generated meet specific set expectations
- External QC
  - Participation in external proficiency programs (human or veterinary)
  - All participating laboratories analyse a common material
  - Comparison of closeness of individual laboratory results to the group mean
  - Provides added assurance that internal processes are consistent with those of the industry at large

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Instrument Validation

- Validation of new instruments to establish/ensure the suitability of instrument and associated reagent systems
  - satisfactory function
  - critical operating characteristics: stray light, zeroing, electrical levels, optical alignment, and background checks
- Biannual calibration according to the manufacturer's instructions
- More frequent calibrations:
  - Following a major service, when quality control values are outside limits, or when workload, equipment performance, or reagent stability indicate the need for more frequent calibration
  - After calibration, controls should be run according to SOPs
  - Instrument validation is closely tied to method validation

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Method/Assay Validation

- Assessing the performance characteristics of an assay
- Requires the selection of the intended biological matrix, including the potential effects of interfering substances and dilution.
- Ensure that performance conforms to the standards of the laboratory and claims of the manufacturer.
- Evaluations may include:
  - Accuracy, Precision
  - Sensitivity, Specificity
  - Total Error
  - Calibration Curve
  - Lower and Upper Limit of Quantification (LLOQ and ULOQ, respectively)
  - Limit of Detection (LOD)
  - Dynamic range (range of linearity)
  - Analyte Stability

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"Fit-for-Purpose" Method/Assay Validation

- Rigor of assay validation depends on intended use of assay:
  - research, drug discovery, animal model development, efficacy, nonclinical, or clinical studies (ranging from exploratory biomarkers to surrogate biomarkers)
  - May be limited to preliminary validation that demonstrates the performance of the assay, yet minimally impacts resources and the drug development timeline
- Factors to consider:
  - Supported studies (nonclinical or clinical)
  - Sample matrix
  - Anticipated range of the variable or analyte in the assay
  - Potential interfering substances in the matrix
  - Acceptable level of variability in the results
  - Availability of reagents and technology

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Over-Interpretation of CP Changes

- Overconfidence in numbers
  - CP interpretation requires familiarity with abnormal values, specimen quality standards and deviations in quality control, and the ability to investigate potential artifacts using all available documentation
- Statistics:
  - Useful aid to:
    - identify mathematical differences between control and test article-treated groups
    - to characterize a trend in the data that may be related to dose and/or duration
  - Supplement rather than replace the process of data interpretation
  - Given a constant N (number of animals), Power (significance, p-value) varies from one analyte to the next
  - Individual animal variability and the low number of animals in non-rodent animal studies complicate the statistical analysis of the data
  - Not all statistically significant changes are test article related
  - Not all test article-related changes are statistically significant

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Biological Significance/Qualification

- What does the analyte value tell us?
  - Indicator of function?
  - Indicator of cellular structural integrity?
- What can cause it to increase or decrease?
  - Clinical Differential Diagnoses
  - Pre-Analytical and Analytical Artifacts/Variability
    - Procedure-related (repeated blood collections, handling, anesthesia)
    - Age/Species/Sex-related
- Based on:
  - known cell biology and pathophysiology
  - long history of diagnostic use in the clinical setting
  - biomarker qualification studies comparing to gold standard

CP Data Interpretation

- Verification of data accuracy (specimen collection/handling records, assay validations, instruments/assay QC records)
- Knowledge of inherent analytical characteristics
- Comparison to concurrent controls, individual animal baseline values and changes over time, and study control range
- Evaluation of individual AND group mean data
- Interpretation of the pattern of changes, rather than the qualitative or quantitative change of an individual analyte, improves meaningful data interpretation and understanding of the relevance of findings.
- Integrated assessment of clinical pathology data in the context of other other available study data: exposure and metabolism data, in-life observations, pathology findings, and, when known, anticipated pharmacology of the test article.
- Case-by-case basis using a “weight-of-evidence” approach

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CP Data interpretation should be performed by a qualified individual

- "Each individual engaged in the conduct of or responsible for the supervision of a nonclinical laboratory study shall have the education, training, and experience, or combination thereof, to enable the individual to perform the assigned functions." (FDA GLP Regulations 21 CFR Part 58)
- Toxicologic pathologist-derived best practices concur that a study pathologist or scientist generating or interpreting data for GLP toxicology studies "must have the education, qualifications and experience to perform these tasks and to integrate pathology with clinical signs, exposure information and other study information." (Morton, 2006)

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CP Data interpretation should be performed by a qualified individual

- A veterinary clinical pathologist has the unique set of education, training and skills required to perform this task
  - Can provide valuable insight beyond stating statistically significant increases/decreases or correlations with histologic lesions.
  - Integration of in-life findings and anatomic pathology findings
  - Findings without AP correlates: functional alterations, acid-base, homeostasis, fluid balance
  - Importance/significance of the findings in animal safety studies
  - Pathophysiology, Mechanism(s) of drug efficacy and toxicity
  - Translation to the Clinic: risk assessment and monitoring
- When possible CP data interpretation should be performed by a veterinary clinical pathologist.
- Limitation: small # of Industry Veterinary Clinical Pathologists

Schultze, A.E., Bounous, D.I. & Bolliger, A.P., 2008. Veterinary clinical pathologists in the biopharmaceutical industry. *Veterinary Clinical Pathology*, 37(2), pp.146–158.

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Veterinary Clinical Pathologists have unique training and knowledge base

- Pre-veterinary coursework or undergraduate degree (BSc)
  - Core: Organic/inorganic chemistry, physics, biology
  - ± embryology, biochemistry, calculus, animal science, livestock judging, animal nutrition, cell biology, genetics
- Veterinary Science Degree (DVM, BVS, BVMS or equivalent)
  - Comparative medicine: anatomy, physiology, biochemistry, pharmacology, pathology, internal medicine, surgery, radiology, microbiology/virology, epidemiology, nutrition
  - Must generally pass medical board examination and be prepared to enter clinical practice as a fully functional animal physician competent in both surgery and medicine
- Residency ± Board Certification in Veterinary Clinical Pathology
  - Formal training in comparative pathophysiology (mechanisms of disease)
  - Specialized training in hematology, hemostasis, clinical biochemistry, urinalysis, cytology, surgical pathology and correlative internal medicine
  - In-depth working knowledge of diagnostic modalities (laboratory instrumentation/assays/methodologies)
  - Proficiency at recognizing and interpreting clinical pathology datasets
- Relevant advanced science degree (MSc or PhD)

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Roles and Responsibilities of the Industry Veterinary Clinical Pathologist

- Clinical Pathologist
  - Review and Interpretation of CP data
  - Clinical pathology peer review
  - Bone marrow and cytology evaluation and reporting
  - ± Supervision/Review/Interpretation of Biomarker data
- Laboratory Support
  - Clinical pathology laboratory management
  - In-house clinical pathology laboratory professional staff training
  - Biomarker development, validation, and implementation
- Clinical Veterinary Diagnostic Support
- Scientific Support
  - Expected variability and sources of variability in the data
  - Quality control and validation of testing methods and laboratory instrumentation
  - Species differences
  - Considerations for study design, proper sample acquisition/handling and biomarker strategies
  - Contribution to the integrated toxicology reports and regulatory documents
  - In-house clinical pathology consultation for discovery, translational, and/or clinical activities
- Drug Safety Leader for Project Teams

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Informal Review of ANY Report is Justified

- Goal: to ensure that test article-related findings are properly identified, consistently diagnosed, and correctly interpreted.
- Fresh Perspective:
  - It is difficult for authors to spot every mistake or flaw in a complicated study
  - Showing work to others increases the probability that weaknesses will be identified and improved.
  - This is not a reflection on the author.
  - An opportunity for improvement may be more obvious to someone with special expertise or with a fresh eye.
- Diversity of Opinion
- Limited Expertise: no one is an expert in everything

Informal Review of Clinical Pathology Reports is Usually Sufficient

- Quality and accuracy of data is ensured by quality control and validation measured that are ensured by SOPs and Deviation Records
- Quality and accuracy of interpretation is ensured by a process involving multiple informal reviews by:
  - AP Peer Reviewer: focus on correlations
    - Formal signed documentation review of clinical pathology interpretation by the anatomic peer-review pathologist is not relevant
  - Study Director: Toxicologists focus on ↑↓numbers and stats
  - Senior Scientific Reviewer: review of integrated report
  - Program/Sponsor Management: focus on adversity, monitorability and translation to the clinic
  - QA Audit: did we follow GLP, QC of interpretive reports
  - ±Internal or External Clinical Pathologists

Common CP Review Comments

- Focus on Numbers
  - It's statistically significant
  - It's not statistically significant
  - It's within the reference range
  - It's only a <10% difference from controls
- Focus on the Familiar
  - Don't recognize effects they have not seen or heard of before
  - Only an increase is significant
  - There's no histologic correlate
  - Not toxicologically/biologically relevant (*adverse? test article-related*?)
  - You can't explain it (we don't know why it's increased/decreased)
- Focus on Formatting and Writing Style
  - "Please always report changes relative to Pretest."
  - "Please only report changes as Fold/Percent."
  - Given vs. Dosed With vs. Administered vs. Receiving

What is Usually Reviewed in an Informal Review of a Clinical Pathology Report?

- CP Data Tables (individual and mean data)
- Interpretive Report
  - Writing style, formatting, calculations, statistics
  - Test Article-Related changes
  - Adverse Test Article-Related changes

What is Rarely Reviewed in an Informal Review of a in a Clinical Pathology Report?

- What rarely gets reviewed or considered, but should:
  - Analytical quality control and validation reports
  - Correlating in-life and AP data and TK
  - Context is sometimes neglected: Study Design and Test article
- What should be reviewed on a case-by-case basis:
  - Peripheral blood or bone marrow smears
  - Specimen collection records
  - Specimen storage conditions (temperature/time)
  - Specimen condition records
  - Analytical run data

Clinical Pathology Peer Review

- Considered separate from AP, SD or Management review
- Should be performed by a qualified individual (Toxicologic/Industry Veterinary Clinical Pathologist)
  - Must have the training and experience to competently evaluate all clinical pathology data within the context of the entire study (Toxicologic/Industry Veterinary Clinical Pathologist).
  - Should be experienced with studies using the same analytes/biomarkers, species and of similar duration and design as the study to be peer reviewed.
  - When appropriate, it may be of value to have several individuals with specialized expertise (hematopoiesis, immunology, etc.) participate in the peer-review process.
  - Experts engaged to consult on specific topics are not required to have the qualifications of a peer-review clinical pathologist, but their contributions should be limited to their areas of expertise.

Adapted from: Morton, D. et al., 2010. Recommendations for pathology peer review. *Toxicologic Pathology*, 38(7), pp.1118–1127.

Clinical Pathology Peer Review

- Performed in consideration of the importance of decisions based on these studies, the experience and skill of the study pathologist, and regulatory requirements
- May be useful in rare instances such as confirmation of unexpected Peripheral Blood or Bone Marrow smear findings
- For CP findings that are critical to decisions regarding NOEL/NOAEL
- When important risk assessment or business decisions will be based on clinical pathology findings in nonclinical studies

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- Adam Aulbach – MPI Resarch
- Peter Mann – EPL
- Jerry Hardisty - EPL

It Takes a Village

- Ideally: good communication between SD, CP and AP produces a quality integrated report.

References on Laboratory Quality Control

- Freeman, K.P. et al., 2006. Introduction to ISO 15189: a blueprint for quality systems in veterinary laboratories. *Veterinary clinical pathology / American Society for Veterinary Clinical Pathology*, 35(2), pp.157–171.
- Westgard, J.O., 2010. Managing quality vs. measuring uncertainty in the medical laboratory. *Clinical chemistry and laboratory medicine : CCLM / FESCC*, 48(1), pp.31–40.

References on Bioanalytical Method (Biomarker) Validation

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