

Risk Assessment and Quality Plans for Next-Generation Sequencing (NGS) assays in the context of evolving regulatory frameworks

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1. Background Information

Next generation sequencing (NGS) technologies enable high-throughput sequencing and play an essential role in the operational implementation of personalized or precision medicine approaches. NGS-based assays are generally developed and offered by clinical laboratories as Laboratory Developed Test (LDTs). LDT testing services and procedures are central to public health and to support medical decision-making. Clinical laboratories offering LDTs operate under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations, which require a laboratory to have quality control (QC) procedures to monitor the accuracy and precision of the complete testing process. Modernization of the CLIA oversight framework in relation to high complexity LDT services is currently under discussion and the U.S. Food and Drug Administration (FDA) is re-evaluating its enforcement discretion approach to LDTs. The Centers for Medicare & Medicaid Services (CMS), the College American of Pathologist (CAP), the American College of Medical Genetics (ACMG), the American Medical Association (AMA), the Association for Molecular Pathology (AMP) and other organizations are key stakeholders in this complex process of changing regulatory frameworks.

Here we present and discuss examples on applying risk assessment strategies to quantify and contain risk in NGS platforms and assays, with the ultimate goal of improving patient testing safety and quality systems frameworks.

Please Note: the Risk Assessment and Approach here presented and discussed is unrelated to Individualized Quality Control Plan (IQCP) regulatory considerations. Specifically, an IQCP option is now available to provide an opportunity to tailor QC plans to the clinical laboratory's unique testing environment, although it is currently un-applicable to NGS clinical laboratories (Refer to the 12/28/2015 clarification to CAP by CMS regarding the unavailability of the IQCP option for NGS molecular-based testing).

2. Methodology

A hybrid approach based on CDC / CMS, CAP / ISO 15189 and Clinical Laboratory and Standards Institute (CLSI) EP23 guidelines and worksheets is used. First, assay process maps are generated to identify risk points, sources of potential failures, or errors in the testing process (Refer to Section 3a, 3b, 3c).

Key questions are to be answered at each processing stage and stages junctures:

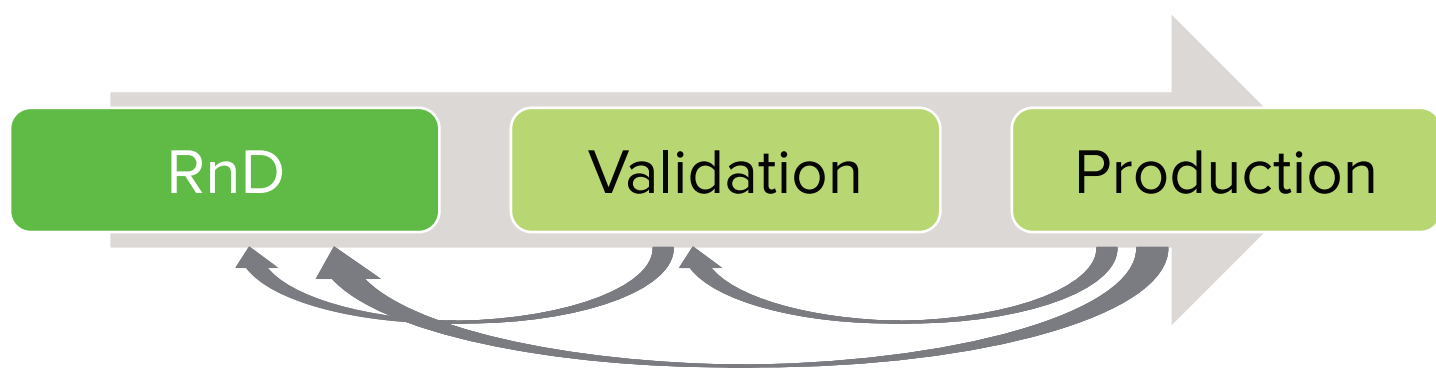
- What can go wrong?
- What are the potential sources of errors?

This includes but is not limited to Risk Assessment in relation to specimens, test systems, reagents, environment, testing personnel, software and LIMS, communications and regulatory compliance (Refer to 4). Risk quantification consists of assessing and grading probability and severity (Refer to 5). Means of failure detection are then charted through a CLSI EP23 modified document in relation to different types of controls (Not Shown). Based on Risk Acceptability and Mitigation determinations, Corrective and / or Preventive CAPA (Corrective And Preventive Action) actions are then initiated and logged into the CAPA Quality System (Refer to 6). In addition, residual risk is placed in the context of a Quality Control (QC) plan and actions taken or planned to mitigate risks. QC plans are evolved to include steps assuring accuracy and reliability of test results in relation to patient care (Refer to 7). Processes and procedures are then modified to eliminate or reduce identified risks at acceptable levels.

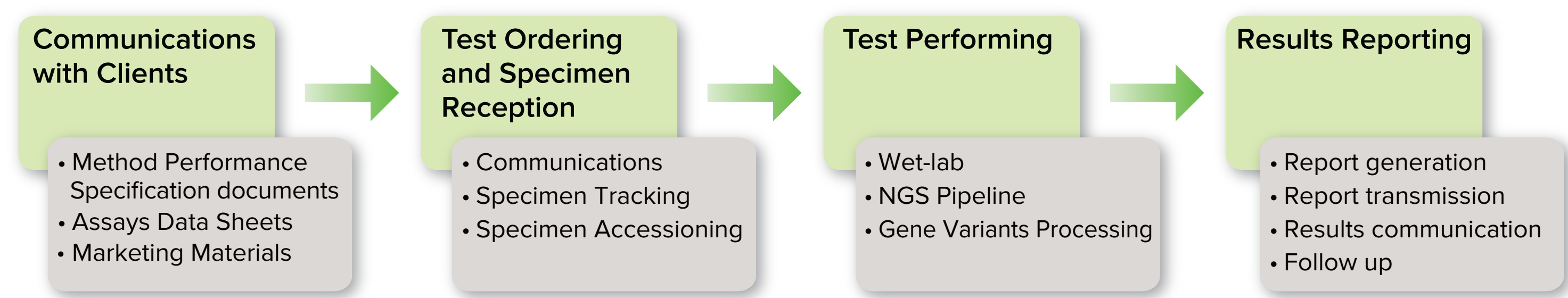
Ongoing Quality Assurance (QA) assessment is performed through Quality Monitors (QMs) and Indicators (QIs) to assess quality based on prior risk assessments and implementations. QMs and QIs monitoring allows for tuning of QC metrics and Standards Operating Procedures (SOPs) steps and new CAPA items are initiated as applicable (Refer to 6).

3. Risk Points Identification and Process Maps (Simplified Versions)

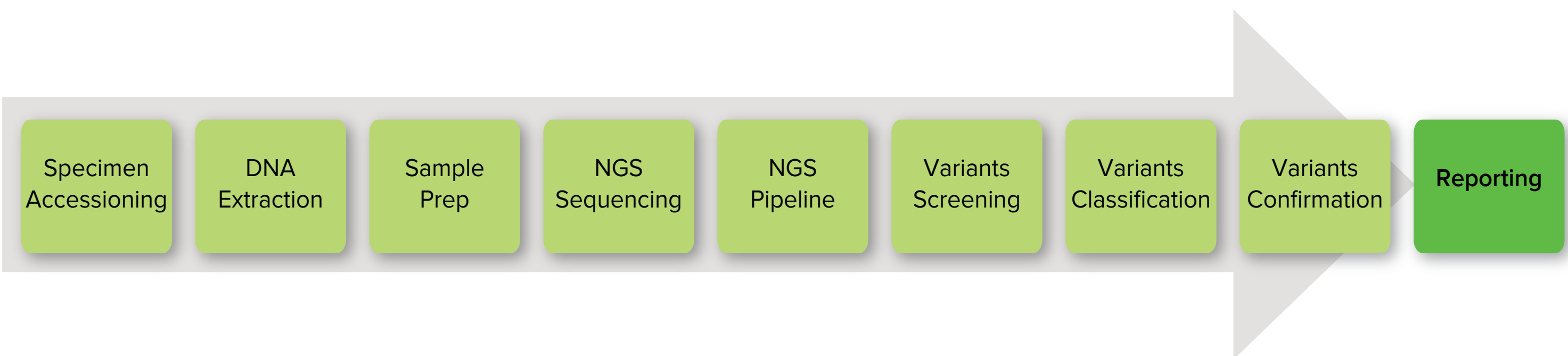
3a. Product (Assay) Life Cycle Map (not comprehensive)



3b. Process (Assay) Map (not comprehensive)



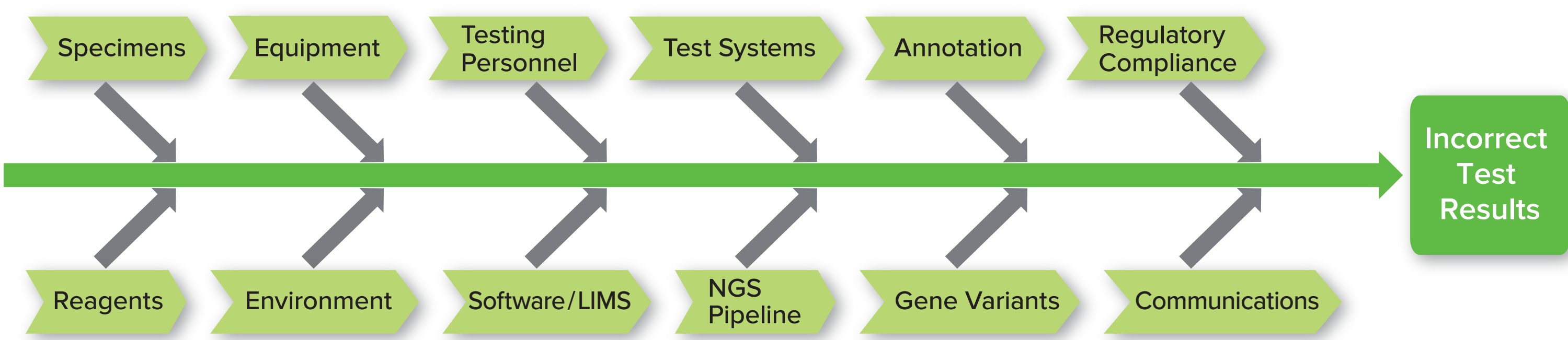
3c. Test (Assay) Performing Map (not comprehensive)



References

- Clinical and Laboratory Standards Institute (CLSI) Guideline EP23 documentation
- College of American Pathologists (CAP) checklists
 - COM.50300 Risk Assessment
 - COM.50600 Quality Assessment Monitoring
- CAP ISO 15189 – "Risk Management Guideline for Laboratories" guideline document

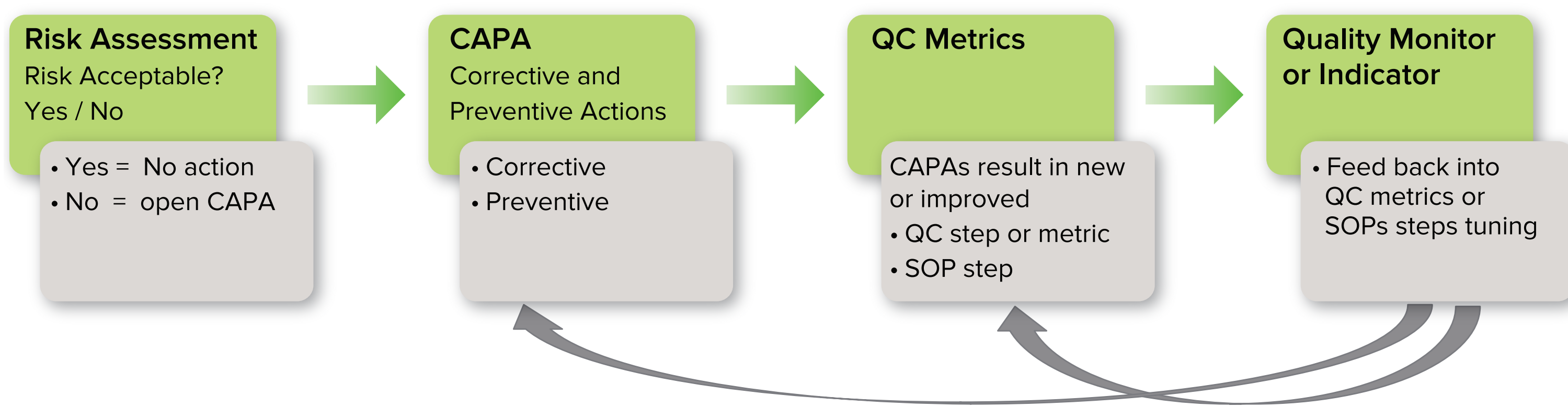
4. Source of Errors Identification through Fish(-Like) Diagram



5. Risk Quantification and Prioritization (Based on CLSI EP23)

Probability of Harm	Severity				
	Negligible	Minor	Serious	Critical	Catastrophic
Frequent	unacceptable	unacceptable	unacceptable	unacceptable	unacceptable
Probable	acceptable	unacceptable	unacceptable	unacceptable	unacceptable
Occasional	acceptable	acceptable	unacceptable	unacceptable	unacceptable
Remote	acceptable	acceptable	acceptable	unacceptable	unacceptable
Improbable	acceptable	acceptable	acceptable	acceptable	acceptable

6. Risk Assessment, CAPAs and Quality Management Plans



7. Examples of Risk Assessment and Risk Mitigation in Relation to Quality Control Plans

Risk Assessment and Mitigation, and related Quality Plans extend to all testing phases (i.e. pre-analytical, analytical, post-analytical, non-phase specific)

Communication (not comprehensive list)

- Quality Goals and / or QC Metrics
 - To provide an informed framework for Clinical Clients (i.e. healthcare providers) regarding Clinical Test availability, Specimen requirements and tests Methods Performance Specifications
- Example of Risks
 - Ordered tests are not clinically indicated
 - Patients and Health Care Providers do not understand test limitations
- Means of Minimizing Risk
 - SOPs; Document Control tools; Change Control Policies

Specimen tracking (not comprehensive list)

- Quality Goals and / or QC Metrics
 - To provide a Specimen tracking and handling controlled process
- Examples of Risks
 - Lost specimens
 - Delays in testing
- Means of Minimizing Risk
 - SOPs; communication; LIMSs workflows; tracking tools

NGS Pipeline (not comprehensive list)

- Quality Goals and / or QC Metrics
 - To define NGS pipeline specifications
 - To define NGS pipeline operation requirements to dedicated staff
 - To define post-pipeline QC metrics
 - To define Validation (major vs minor) requirements
- Examples of Risks
 - Lack of NGS Pipeline new releases proper Verification and Validation
 - Passing of operation sequencing runs not meeting required QC metrics
 - Lack of / insufficient training and competency for staff running the NGS Pipeline
- Means of Minimizing Risks
 - SOPs; LIMSs workflows; proper staff training and competency

Conclusions

Our work and experience suggests that integrated Risk Management approaches may assist in de-convoluting LDTs NGS-based assays complexity.

Risk Management encompasses Risk Identification and Assessment; Risk Quantification; Risk Control, Mitigation and Acceptance; Risk Communication; and Risk Monitoring. Risk Management is operated through dedicated teams of ad-hoc stakeholders.

This Risk Management integrated approach is expected to result in an improvement of NGS QC plans in relation to key process steps and critical factors such as annotation, databases, software, testing personnel competency, reagents and environment.

Integration of risk management strategies and QC / QA plans with everyday testing observations, Proficiency Testing (PT) analysis, and assessment of new assay validations provides the basis to maintain, extend and evolve dynamic QA frameworks.