



Quality Control and Assurance in ISO15189:2012 Molecular Cytopathology Laboratories for HPV DNA Testing

Eleftherios Vavoulidis*, Maria Nasioutziki, Evangelia Mareti, Vasiliki Karpa, Nikolaos Tsabazis, George Chrysostomos Pratilas, Anastasios Liberis, Fausto Carcea, Stamatios Petousis, Angelos Daniilidis and Konstantinos Dinas

Molecular Cytopathology Laboratory, 2nd Obstetrics and Gynecology Department, Medical Faculty, Aristotle University of Thessaloniki, Greece

***Corresponding Author:** Eleftherios Vavoulidis, Molecular Cytopathology Laboratory, 2nd Obstetrics and Gynecology Department, Medical Faculty, Aristotle University of Thessaloniki, Greece.

Received: October 28, 2019; **Published:** December 03, 2019

DOI: 10.31080/ASMI.2020.03.0445

Abstract

Molecular cytopathology laboratories offer an outstanding portfolio of testing services that are vital for healthcare including, among others, HPV DNA testing for cervical cancer screening. Due to their clinical importance, such laboratories have to provide credible and reproducible testing results. Accreditation status and the implementation of a Quality Management System according to ISO15189 requirements are usually the most effective strategies to reach the optimum quality standards. However, laboratory quality should be constantly monitored and evaluated in order to avoid potential deviations from the pre-defined quality standards. The design of a unified system of Quality Control and Assurance procedures that will guarantee excellent laboratory performance is essential. The authors present their experience on the implementation of such a system and describe in detail strategies for efficient laboratory quality management while making useful recommendations and underlining vital key points.

Keywords: Quality Management System; Quality Control and Assurance System; Inter-Laboratory Schemes; Molecular Technique Verification; Annual Management Review

Abbreviations

QC: Quality Control; QA: Quality Assurance; HPV: Human Papilloma Virus; QCAS: Quality Control and Assurance System; QMS: Quality Management System; LOD: Limit of Detection; M-LOD: Manufacturer's LOD; L-LOD: Laboratory LOD

Introduction

Various definitions can be used to describe the term "quality", especially in the field of healthcare and medical services. Quality can be defined as the threshold below which the final product or service is insufficient or inadequate to satisfy the needs of the consumers/users. So, in terms of Molecular Laboratory Testing, quality is the group of characteristics and attributes that a specific diagnostic service or product should have in order to satisfy the

stated needs and the implied expectations of the referral doctors and patients requesting them [1].

Molecular Cytopathology laboratories offer diagnostic services that are crucial for patient care. The introduction of liquid-based cytology (LBC) have enriched modern cytopathology laboratory with an outstanding testing portfolio that has been extended from the cell observation to the analysis of DNA and RNA molecules, genetic markers and proteins [2,3]. Human Papilloma Virus (HPV) DNA tests that use molecular methodologies to detect the presence of genetic material from numerous HPV subtypes, the main etiologic factor for cervical cancer [4], consist the one of the most important testing service a molecular cytopathology lab can offer to gynecologists and patients [5,6]. All this molecular testing include

arrangements for examination requests, patient preparation and identification, collection, transportation, storage, processing and evaluation of clinical samples, together with subsequent interpretation, reporting and advice.

Due to the high clinical significance of their services, Molecular Cytopathology laboratories need to develop and apply strategies ensuring that the quality of the laboratory performance will always be adequate to satisfy the needs of the patient care while being suitable for its intended use. The most efficient way to achieve laboratory quality is through the design and implementation of a Quality Management System (QMS) that fulfills the requirements of the ISO15189:2012 International Standard for medical laboratories and by obtaining accreditation status from a committee of external peer-reviewing experts [7,8]. ISO15189 is an ideal and well-designed combination of the quality system requirements of ISO9001 with the competency requirements of ISO17025 that focuses, among others, on critical pre-analytical, analytical and post-analytical processes that may affect the quality of the provided laboratory services while addressing the specific needs of diagnostic laboratories including the cytopathology ones [9].

A modern diagnostic laboratory should not only aim to provide high quality services to its clients but also monitor constantly its performance and try to maintain or even enhance its quality standards. In other words, once laboratory quality is initially achieved, the laboratory should take all the necessary measures to ensure that the established quality values will be high at any given time. This is achieved mainly by Quality Control (QC) and Quality Assurance (QA) procedures that compose a well-structured Quality Control and Assurance System (QCAS) that a diagnostic laboratory should implement in its well-structured QMS. QC defines service's quality, imparting to it the credibility needed for its intended purpose, while QA activities measure the degree to which desired outcomes are successful [10]. The aim of this article is to depict our experience on the implementation of a QCAS into the already established ISO15189:2012 QMS. The overall internal QC and external QA procedures that our molecular cytopathology lab has applied and follows, are analytically described. In addition, helpful recommendations and useful suggestions are made in order to help other cytopathology laboratories in initially designing or further improving their QCAS by making it more efficient without drifting away from the ISO15189:2012 requirements.

Laboratory quality control and assurance in molecular cytopathology examinations

A laboratory QMS based on the requirements of ISO15189:2012 should include well-demonstrated and well-implemented QCAS that will guarantee that the laboratory performance and results satisfy the pre-defined quality standards.

Participation in external QA inter-laboratory scheme

A modern cytopathology laboratory, whose testing portfolio is not only limited to morphological examinations but is extended to molecular ones, should participate in external QA schemes suitable for evaluating the laboratory's performance. After proper evaluation of all the potential providers of external QA schemes, our Laboratory Management decided to participate into two external QA programs: one organized by UK NEQAS and one by World Health Organization (WHO). UK NEQAS is the national external QA service provider of United Kingdom that offers more than 390 QA programs covering every possible field of laboratory medicine while WHO HPV Lab Net has developed an international proficiency panel for HPV DNA detection and typing, and organized annual proficiency studies since 2007. The UK NEQAS scheme has a testing panel of 12 LBC clinical specimens while the panel of WHO HPV Lab Net scheme is composed of 43 samples (purified whole genomic plasmids) of high/low-risk HPV types in a background of human DNA, and 3 extraction controls.

Upon receipt of the final results, the findings of the report are reviewed by the Laboratory Director and the Quality Manager in order to assess the performance of the molecular biologists of the laboratory. Then, the involved scientific staff is informed about their performance score and other important data that is extracted from the assessment reports. In cases of low performance scores that result in crucial deviation from the mean performance of other participating laboratories, the laboratory personnel will arrange a meeting where all the essential corrective and preventive measures in order to fulfill its predefined quality and performance criteria will be designed and applied as soon as possible.

Molecular re-testing of already analyzed LBC specimens

The laboratory has implemented an internal QC system that involves a second HPV DNA testing of already diagnosed LBC samples that are stored in the laboratory facilities. In particular, the molecular biologists have to re-analyze at least 10% of the total LBC sam-

ples they evaluate annually. This internal QC system is consisted by two main review categories:

- The intra-observer category where every molecular biologist retest LBC samples they have already diagnosed earlier) in order to evaluate the accuracy, diagnostic agreement and reproducibility associated with their personal performance.
- The inter-observer category where every molecular biologist is asked to analyze LBC specimens previously examined by their colleagues in order to evaluate the accuracy, diagnostic agreement and reproducibility among all the laboratory molecular biologists.

The selection of the LBC samples for re-analysis is carefully done in a way to include as many clinical cases that represent the every-day workflow. In addition, LBC samples that have revealed a co-infection of various high-risk HPV genotypes, especially when HPV-16 and HPV-18 are included, are considered as an ideal choice for re-analysis. Also, LBC specimens that have been diagnosed as negative for HPV genotype while the corresponding Pap smear showed low or/and high-grade intraepithelial lesions could be a promising and interesting option for molecular re-evaluation.

In order to determine the diagnostic agreement and accuracy of a molecular biologist, the laboratory do not necessarily need to use as reference only the testing performance of its other working biologists. Hence, the laboratory can perform its molecular QC process by recruiting the biologists of its referral laboratories who will be asked to evaluate a selection of LBC samples.

The laboratory carries out this internal QC review every month in order to constantly monitor its personnel diagnostic performance and continuously check for potential non-conformities that could question the quality of the provided molecular testing services. The Quality Manager has compiled specific files where each LBC sample that has been re-analyzed is reported along with the diagnostic evaluations both the initial and the reviewing one as well as the identity of the biologist who carried out each evaluation.

Statistical analysis

The molecular examinations of the LBC samples are properly categorized according to known different severity level of the HPV types according to the international literature [11-14]. In particu-

lar, the laboratory categories its molecular findings in the 7 following categories:

- Category 1: Samples characterized as inadequate/insufficient for molecular evaluation.
- Category 2: Samples characterized as negative for HPV genotypes.
- Category 3: Samples where HPV detection is uncertain (possibly due to small viral load).
- Category 4: Samples where low-risk HPV types are detected (LR-HPV).
- Category 5: Samples where probably high-risk HPV types are detected (prHR-HPV).
- Category 6: Samples where high-risk HPV types are detected (HR-HPV).
- Category 7: Samples where either or both the ultra-high-risk HPV-16 or/and HPV-18 are detected (uHR-HPV).

The results that are generated from the statistical analysis are evaluated in terms of performance assessment of one particular biologist or/and in terms of performance comparison between different biologists. Diagnostic reproducibility of the HPV DNA testing, is measured by the kappa statistic. Within the positive kappa values, the agreement was interpreted as follows: a range of 0.00–0.20 indicates slight agreement, a range of 0.21–0.40 indicates fair agreement, a range of 0.41–0.60 indicates moderate agreement, a range of 0.61–0.80 indicates very good agreement, while a range of 0.81–1.00 indicates excellent or almost perfect agreement [15].

Correlation between molecular evaluations and other examinations

The laboratory has applied a procedure for comparison of each HPV DNA test with cytological and histological examinations to which the same patient was subjected to during that period of time, when this is possible, in collaboration with the Cytologists and Pathologists of the Hospital. The Laboratory Manager has composed specific files where each patient is reported along with the diagnostic findings that were generated from the corresponding cytological, histological and molecular analysis.

This way it is possible to distinguish molecular diagnoses that are in agreement with the corresponding cytological and/or histological examinations from others that are not. This correlation process is very important since it acts as a powerful comparison

tool when trying to choose which LBC samples may be interesting selections to be included in the previously mentioned re-analysis of HPV DNA testing cases. More specifically, LBC samples that are positive for high-risk HPV genotypes (especially in cases of HPV-16 and HPV-18) while their cytological and histological findings are negative, are usually considered as good choices as well as LBC samples whose cytological and histological diagnoses show low or high-grade intraepithelial lesions while no HPV DNA was detected.

In-house verification of the CE IVD molecular analytical technique

Modern cytopathology laboratories use mostly diagnostic kits that are marked as “CE-IVD” (Conformité Européenne – *In Vitro* Diagnostics). The European Union’s Directive on IVD Medical Devices claims that an IVD product is manufactured under excellent conditions in agreement with strict quality requirements that ensures that this product achieves the stated performance and will continue to perform as stated when used for relative analyses in any diagnostic laboratory in the world. In particular, most manufacturing companies that produce diagnostic kits based on molecular biology principles are accredited according to ISO 13485: Medical devices – Quality Management whose requirements and clauses significantly boost the quality standards of the final diagnostic kit. All the analytical techniques of this category are considered as validated which means that the manufacturers constantly assess their quality characteristics through experimental processes to verify whether they are in accordance with the pre-defined product specifications and fit for their purpose of use or not [10].

Nevertheless, the ISO 15189:2012 dictates the need for in-house verification of any CE IVD analytical method that is used for molecular evaluation in a cytopathology laboratory even if it has been validated by its manufacturer. This basically means that the laboratory should design and implement a procedure that will demonstrate that the used methodology for molecular evaluations is adequate to provide precise and accurate results and is suitable for the intended use of the laboratory at any given time. More specifically, the laboratory has to evaluate the following parameters associated with the used analytical technique for HPV testing.

Trueness: It is defined as the degree of agreement of a measured or calculated quantity with its true value. It is also known as “accuracy” and is expressed as the level of conformity in a series of molecular evaluations of reference samples or clinical samples

with verified diagnosis with the same equipment and devices. These series of evaluations can be carried out during the same or/and different working days [16-18].

In detail, to evaluate trueness the laboratory analyzed a series of samples either positive or negative for HPV genotypes. These samples were either reference samples used in UKNEQAS/WHO QA schemes or clinical samples that have been diagnosed with other alternative HPV DNA techniques. Trueness was calculated from the ratio of the total true positive and true negative samples to the total number of analyzed samples. In addition, further evaluation of trueness was achieved by including the results from UKNEQAS/WHO inter-laboratory schemes as well as results from recovery experiments.

Repeatability: It is defined as the level of concordance between specific diagnostic evaluations of the same sample performed within the same experimental run. It is also known as “intra-assay precision” and is expressed as the level of diagnostic agreement in a series of molecular evaluations as an outcome of multiple repeating analyses of the same specimen with the same equipment under the same experimental conditions (same biologist) during one particular working day [16-18].

More specifically, the laboratory calculated the repeatability value by analyzing numerous samples either negative or positive for HPV genotypes at least 3 times during a particular experimental run.

Reproducibility: It is defined as the level of concordance between specific diagnostic evaluations of the same sample performed during a series of experimental runs. It is also known as “inter-assay or inter-mediate precision” and is expressed as the level of diagnostic agreement in a series of molecular evaluations as an outcome of multiple analyses of the same sample under different experimental conditions (if possible different equipment and different biologist) during different working days [16-18].

Reproducibility was assessed by analyzing each sample either negative or positive for HPV genotypes at least 6 times during different experimental runs that were carried out on different working days.

Limit of Detection (LOD): It is defined as the lowest quantity of nucleic acid per specimen that can be detected during the experimental analysis. LOD during HPV DNA testing, is expressed as the minimum quantity of HPV DNA per sample that is possible to be detected at least in the 95% of the total evaluation that are carried out.

The laboratory had to evaluate whether the LOD values of the diagnostic kit provided from the manufacturer (m-LOD) can be achieved and verified by the laboratory personnel [16-18]. In other words, the scientific staff had to design and execute a series of molecular DNA analyses in order to calculate precise and accurate values of laboratory LOD (l-LOD) and correlate the agreement between l-LOD and m-LOD values. In detail, for each HPV genotype, a number of particular dilutions were created with concentrations close to the corresponding m-LOD. More specifically, the laboratory produced control samples with overall HPV concentration that was either less or more than the established m-LOD by 10%, 20% and 30% respectively. Also, controls with viral concentrations equal to m-LOD were produced and analyzed as well. Each diluted sample was analyzed at least 5 times. The generated l-LOD values were compared with the provided m-LOD values for each HPV genotype in order to assess the level of agreement between them.

It is worth mentioning that for the entire in-house validation, the diluted samples that were used as positive reference controls during the experiments, were either HPV DNA samples purchased from the WHO (First International Standards) or clinical samples that were previously used in the UK NEQAS and WHO Labnet QA schemes. Certified negative clinical samples from the same QA schemes were used as negative reference materials. Of course, it not possible for a cytopathology laboratory to possess a portfolio of certified reference materials for each of the numerous HPV genotypes that are detected by the applied molecular technique, but at least a satisfying number of unique positive controls should be used.

This entire validation process of the established molecular technique is not static but rather dynamic. More specifically, the laboratory after the successful accomplishment of the extensive and in-depth initial validation of its accredited molecular technique, performs a periodic mini-retest of the achieved validation.

Apart from the constant monitor of the validation process, this procedure acts as an additional QC strategy that verifies the quality standards of the purchased reagents that are going to be used. Furthermore, the selection of the reference materials that are going to be re-analyzed each time is assigned to a laboratory member different than the analyst that will perform the testing in order to ensure the credibility and objectivity of the process. Also, it is possible to create a new control material with proper mixing of various controls each one positive for different HPV genotype mimicking this way a reference sample that simulates a clinical case of a multiple HPV co-infection [19].

Detailed documentation of experimental evaluations

The laboratory keeps very detailed experimental notebooks where the biologists record all the experimental conditions, analytical data and testing notes. The laboratory keeps separate notebooks for each one of its molecular examinations. A unique serial number is assigned on every notebook. Also, the pages of all the laboratory notebooks have to be numbered and dated. Additionally, the laboratory keeps two specific files: one associated with the process of DNA extraction from the biological samples and one associated with the PCR amplification of the patient DNA extracts and the following HPV detection process. Both lab-books, apart from the previously described specimen parameters and information, include some process-specific information. In particular, the extraction lab-book contain the DNA total concentration and A260/A280 ratio values of the generated DNA extract that are calculated via a spectrophotometer and represent the quality and quantity of the extracted DNA. On the other hand, the PCR lab-book includes the final HPV genotyping results. Both lab-books are signed by the molecular biologist that performed each experimental run while the lot/batch number of the diagnostic kit and reagents used each time are reported as well. Only authorized staff is allowed to fill in these notebooks. The information contained in the printed lab-books is also kept in separate digital files from the Quality Manager for easier and faster tracking of a specific analysis and for backup reasons as well. Furthermore, the notebooks have to be kept in excellent condition since they are regarded as an important item for inspection during the internal laboratory audits that take place periodically.

Internal audits

The laboratory has integrated into its established QMS a specific procedure associated with an internal auditing system. These

internal audits, apart from evaluating the level of laboratory commitment to the ISO 15189:2012 management and technical requirements [19], they assess the level of technical competency and diagnostic performance of the laboratory personnel acting as an additional QC and QA in-house strategy. These internal audits can be vertical ones during which a detailed evaluation of a particular testing process is performed including all its relative pre-analytic, analytic and post-analytic stages. During a vertical audit, the corresponding auditor can evaluate the overall analytical performance and technical skills of a particular laboratory member by monitoring for example the experimental analysis and molecular evaluation of a number of LBC samples by a laboratory molecular biologist.

The Quality Manager has composed a specific document report that includes a detailed and analytic questionnaire and a checklist associated with the items that were evaluated during each vertical audit in order to help the auditors when reporting their findings. It should be mentioned that not only scheduled vertical audits are included in the internal audit program. The laboratory is capable to conduct an unscheduled internal audit without previous notice of the laboratory personnel that is going to be assessed.

Annual review of the internal QC and external QA schemes by the management

At the beginning of the year, the laboratory arranges a meeting where review by the Management is done. During this review, among others, the Laboratory Director and the Laboratory Manager have to evaluate all the important information and data extracted from the QC and QA laboratory activities during the previous year. The results from this in-depth analysis are discussed with the personnel and other associates at this meeting,

In terms of internal QC laboratory procedures, the Management evaluates the reports associated with the molecular re-analysis of the LBC samples during the previous year. Correlation between the initial diagnosis and the second one is determined with statistical tools, usually expressed with Kappa statistic values [20] and depicted with Receiver Operation Characteristic (ROC) curves [21,22]. Kappa statistic values and ROC Curves consist one of the most important quality indicators that the laboratory should determine for its molecular examinations. Important deviations observed between the two diagnoses are reported in order to be further investigated by the laboratory personnel. Also, the overall

performance of the working biologists is properly assessed by calculating the diagnostic agreement both intra- and inter-observatory that are generated from the entire re-analysis process.

In terms of the inter-laboratory QA schemes, the Management analyzes and evaluates the findings included in the final reports from the QA scheme providers in order to verify whether the overall laboratory performance of molecular biologists was equal or close to the performance standards defined during the last year's annual meeting or not. Statistical correlation between the diagnostic results submitted by the laboratory staff and the intended ones provided from the QA scheme organizers is also expressed through Kappa statistic values [20] and depicted with ROC curves [21]. When important non-conformities or deviations are detected, the laboratory should take immediate corrective and/or preventive action adequate to restore the staff performance to the desired optimal level. Such action can include among others, extensive re-training of the scientific personnel, new working instructions, possible changes in established procedures, potential equipment replacement etc. In some cases, the laboratory may contact the scheme provider or a collaborating laboratory advisor for valuable assistance and complementary information that could significantly help in the design of the corresponding action-plan [19,23].

At the end of the meeting, the laboratory should set new quality indicators associated with the QCAS during the current year with focusing on maintaining or even increasing its Kappa statistic values in both internal QC and external QA procedures in order to continue to provide high quality testing services to doctors and patients.

Conclusion

The third edition of ISO 15189 has been enriched with additional requirements both technical and management that will assist molecular diagnostic laboratories including the cytopathology ones to provide excellent testing services to healthcare. A well-designed and well-structured QCAS as an inner part of the total established QMS is necessary for proper and continuous monitoring of the quality standards that characterize the laboratory testing at any given time. Strict measures and efficient strategies have to be applied in order to maintain or even improve the quality of the laboratory performance. Molecular cytopathology labs have to create an ideal combination of internal QC and external QA procedures that will cover their extended testing portfolio ensuring high quality standards.

Conflict of Interest

There is no conflict of interest

Bibliography

1. Branca Margherita and Adhemar Longatto-Filho. "Recommendations on Quality Control and Quality Assurance in Cervical Cytology." *Acta Cytologica* 1 59.5(2015): 361-369.
2. Salto-Tellez Manuel. "More than a Decade of Molecular Diagnostic Cytopathology Leading Diagnostic and Therapeutic Decision-Making". *Archives of Pathology and Laboratory Medicine* 142.4 (2018): 443-445.
3. Pisapia Pasquale., et al. "Bird's Eye View of Modern Cytopathology: Report from the Seventh International Molecular Cytopathology Meeting in Naples, Italy, 2018". *Cancer Cytopathology* (2019).
4. Walboomers Jan MM., et al. "Human Papillomavirus Is a Necessary Cause of Invasive Cervical Cancer Worldwide". *Journal of Pathology* 189.1 (1999): 12-19.
5. Campos Nicole G., et al. "Estimating the Value of Point-of-Care HPV Testing in Three Low- and Middle-Income Countries: A Modeling Study." *BMC Cancer* 17.1 (2017).
6. Cuzick Jack., et al. "Overview of Human Papillomavirus-Based and Other Novel Options for Cervical Cancer Screening in Developed and Developing Countries". *Vaccine* 26.10 (2008): K29-K41.
7. Alkhenizan Abdullah and Charles Shaw. "Assessment of the Accreditation Standards of the Central Board for Accreditation of Healthcare Institutions in Saudi Arabia against the Principles of the International Society for Quality in Health Care (ISQua)". *Annals of Saudi Medicine* 30.5 (2010): 386-389.
8. Braithwaite Jeffrey., et al. "Strengthening Organizational Performance through Accreditation Research-a Framework for Twelve Interrelated Studies: The ACCREDIT Project Study Protocol". *BMC Research Notes* 4 (2011): 390.
9. Vavoulidis Eleftherios., et al. "Transition to ISO 15189: 2012 for Cytopathology Laboratories Part 1: General and Management Requirements". *International Journal of Reliable and Quality E-Healthcare (IJRQEH)* 5.3 (2016): 1-21.
10. Vavoulidis Eleftherios., et al. "Transition to ISO 15189: 2012 for Cytopathology Laboratories Part 4: Examination Processes and QC / QA System (QCAS)". *International Journal of Reliable and Quality E-Healthcare* 5.3 (2016): 62-82.
11. Bouvard Véronique., et al. "A Review of Human Carcinogens-Part B: Biological Agents". *The Lancet Oncology* 10.4 (2009): 321-322.
12. Bzhalava Davit., et al. "A Systematic Review of the Prevalence of Mucosal and Cutaneous Human Papillomavirus Types". *Virology* 445.1-2 (2013): 224-231.
13. IARC. "Monograph on Human Papillomaviruses." IARC Monographs on the Evaluation of Carcinogenic Risks to Humans – WHO 90 (2007): 46-179.
14. Ghittoni Raffaella., et al. "Role of Human Papillomaviruses in Carcinogenesis". *Ecancer medical science, Cancer Intelligence* 9 (2015).
15. McHugh Mary L. "Interrater Reliability: The Kappa Statistic". *Biochemia Medica* 22.3 (2012): 276-282.
16. Rabenau Holger F., et al. "Verification and Validation of Diagnostic Laboratory Tests in Clinical Virology". *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology* 40.2 (2007): 93-98.
17. Kessler Harald H and Reinhard B Raggam. "Quality Assurance and Quality Control in the Routine Molecular Diagnostic Laboratory for Infectious Diseases". *Clinical Chemistry and Laboratory Medicine* 50.7 (2012): 1153-1159.
18. Mattocks Christopher J., et al. "A Standardized Framework for the Validation and Verification of Clinical Molecular Genetic Tests". *European Journal of Human Genetics: EJHG* 18.12 (2010): 1276-1288.
19. Vavoulidis Eleftherios., et al. "Transition to ISO 15189: 2012 for Cytopathology Laboratories Part 2: Technical Requirements". *International Journal of Reliable and Quality E-Healthcare (IJRQEH)* 5.3 (2016): 22-41.
20. Andrés A Martín and P Femia Marzo. "Chance-Corrected Measures of Reliability and Validity in K x K Tables". *Statistical Methods in Medical Research* 14.5 (2005): 473-492.
21. Lasko Thomas A., et al. "The Use of Receiver Operating Characteristic Curves in Biomedical Informatics". *Journal of Biomedical Informatics* 38.5 (2005): 404-415.
22. Fan Jerome., et al. "Understanding Receiver Operating Characteristic (ROC) Curves". *Canadian Journal of Emergency Medicine Decker Publishing* 8.1 (2006): 19-20.
23. Vavoulidis Eleftherios., et al. "Transition to ISO 15189: 2012 for Cytopathology Laboratories Part 3: Risk Analysis and Management". *International Journal of Reliable and Quality E-Healthcare (IJRQEH)* 5.3 (2016): 42-61.

Volume 3 Issue 1 January 2020

© All rights are reserved by Eleftherios Vavoulidis., et al.