

A Stakeholders Approach for Curriculum Development of Master's Degree in Molecular Diagnostics

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Background: Curriculum development is a multi-processing activity that involves many academic and professional stakeholders. In order to detect the curriculum components, it is very helpful to determine the needs and expectations of the stakeholders concerning the graduate's competencies. The main objective of this work is to develop a curriculum for a master's degree in molecular diagnostics based on a survey of key stakeholders and according to the requirements of accreditation and certification, while maintaining its relevance with the rapidly advancing diverse techniques.

Methods: Experts and supervisors including professors of molecular diagnostics at the various universities and consultants and supervisors at health-care centers performing molecular testing were surveyed to assess their expected cognitive and psychomotor molecular skills from a master's degree graduate. A validated questionnaire that included demographic information, current practiced molecular techniques, the level of expected expertise, and the educational requirements for each.

Results: Thirty-six respondents, mostly with a doctorate degree and more than 10 years' experience, have successfully completed the questionnaire. More than 60% of the participating laboratories are commonly used or planned to be used within the next five years. About 57.4% required expert and familiar with skills and concepts. In general, the overall score of skills expectations was 2.8 ± 0.0 out of four. The practice level for molecular techniques was in favor of a master's degree (53.8%). The level of skills expectation is very high for the specific managerial and quality activities with an overall value of 3.7 ± 0.3 out of four.

Conclusion: We gathered information on the standard requirements of the professional practice and on its anticipated future directions through surveys and interviews with the professional practitioners and educators to develop a curriculum for a master's degree in molecular diagnostics. The two major messages from the stakeholders are that both cognitive and psychomotor skills of the mentioned molecular techniques are required for the program and there is a need to include extensive laboratory training during the courses.

Keywords: curriculum, stakeholder, molecular diagnostics, cognitive skills, psychomotor skills

Introduction

Molecular tests are highly complex and therefore, require highly trained personnel with a high level of a diversity of skills.¹ These include cognitive and psychomotor competencies in addition to technical analytic management with the associated need for bioinformatics and information technology support.² Thus, there is an increased demand for postbaccalaureate programs that qualify senior laboratory practitioners who can oversee laboratory tasks and ensure the highest level of quality.³

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Many studies indicated that the qualifications of laboratory practitioners, including training and experience, are critical for ensuring quality performance of molecular testing, because human error has the greatest probable effect influencing the quality of laboratory test results.^{4,5} It has been determined that clinical laboratory practitioners with advanced degrees had an increased management authority and had made significant professional contributions as compared to their baccalaureate-level colleagues.^{6,7}

Generally, practice levels and educational needs for clinical laboratory personnel in cytogenetic, advanced molecular, advanced flow cytometry, and histocompatibility require a baccalaureate degree together with additional education and relevant experience. Whereas overall management of the laboratory, quality assurance, process improvement, and information management require a master's degree in the relevant area.⁸⁻¹¹

Curriculum development should follow as much as possible the current knowledge of educational advancements and strategies, following a predetermined, mission, goals, and the selected educational model while considering the accreditation guidelines and the stakeholders' expectations. Curriculum development is a multi-processing activity that involves many academic and professional stakeholders. Academic stakeholders are the primary source of information and define and organize the content, teaching and learning strategies, assessment and evaluation processes into a logical pattern and decide the level of curriculum that qualifies for a certain profession.^{12,13} On the other hand, professional stakeholders have a great concern in specific professions such as graduates' attributes, especially cognitive and psychomotor skills.¹⁴⁻¹⁷

The clinical laboratory science education is being challenged by the introduction of complex molecular diagnostics techniques that were previously performed only in research settings.

Molecular diagnostics is the result of the effective interaction of laboratory medicine, genomics knowledge, and molecular genomic technologies and described as a group of techniques that includes the detection of genomic variants, in order to promote detection, diagnosis, subclassification, prognosis, and monitoring response to therapy.¹⁸ The success of the human genome project, forensic applications, genetic identification of various disease-causing microbes, detection of bioterrorism agents, and expanded public health epidemiology and surveillance activities have all contributed to the rapid incorporation of

molecular diagnostics into the routine practices of medical and public health laboratories.¹⁹⁻²¹

Molecular techniques used in clinical settings are mostly either hybridization or amplification assays. Hybridization assays, including blotting techniques and microarrays, involve the complementary binding of a labeled probe of known nucleic acid sequence with related DNA or RNA molecules derived from the patient sample.²² Nucleic acid amplification testing is a technique that involves amplification and detection of the nucleic acids for diagnosis to provide guidance on therapy.

Clinical applications of nucleic acid testing are manifold, including the detection of genetic diseases, infectious disease, forensics testing, epigenetics, human leucocyte antigen typing, immunotherapy and immunosuppression, metagenomics, molecular endocrinology, molecular oncology, toxicology, coagulation, and pharmacogenomics.

For the success of molecular diagnostics in a clinical setting, it is critical that laboratory workers be well-trained in performing, troubleshooting, and interpreting the assays. They must understand the limitations of both the technology and the obtained results.²³ Therefore, future laboratory practitioners are required to be knowledgeable in the basic principles and applications of molecular diagnostics technology.²¹

In order to detect the curriculum components, it is very helpful to determine the needs and expectations of the stakeholders concerning the graduate's competencies.

The main objective of this work is to develop a curriculum for a master's degree in molecular diagnostics based on a key stakeholder survey and according to the requirements of accreditation and certification, while maintaining its relevance with the rapidly advancing diverse techniques. Experts and supervisors including professors of molecular diagnostics at the various universities and consultants and supervisors at tertiary care centers performing molecular testing were surveyed to assess their expected cognitive and psychomotor molecular skills from a master's degree graduate.

Methods

The Research and Ethics Committee of Prince Sultan Military College of Health Sciences, Dhahran, approved this study (IRB Number IRB-2018-CLS-001). An advisory board consisted of educators, managers, and laboratory professionals, was formed to guide the development of the surveys, review results, and make recommendations.

Laboratory directors of tertiary care centers and university educators were asked to identify individuals

involved in molecular diagnosis. Forty-five participants were selected through this process of snowball sampling. All participants provided written informed consent.

The questionnaire, which was a modification of a previously described questionnaire²⁴ according to the objectives of the study, consisted of several scale choices and open-ended questions. The first part of the questionnaire included demographic information such as qualifications, field of specialty, experience, and address. The second question was to indicate whether the listed molecular methods (22 techniques/procedures) are currently practiced or planned to be performed in the next five years at the corresponding laboratory. The current molecular techniques that represent amplification techniques, enzymatic-based methods, electrophoretic-based techniques, solid phase-based hybridization or blotting techniques, microarrays, sequencing, DNA/RNA isolation, primer design, assay development, and verification were included. Respondents were asked to rate the previously mentioned techniques in terms of expertise levels expected from a laboratory practitioner or a recent graduate by using a four-point Likert scale rating ranging from expert (4), familiar with skills and concepts (3), familiar with concepts (2), and unfamiliar (1).

Another question explored the appropriate level of education for each technique by choosing baccalaureate or master's degree. The last question was to rate special issues of quality control and management in terms of how expert recent graduates of a master's degree program in molecular diagnostics should be by using a four-point Likert scale rating ranging from expert (4), familiar with skills and concepts (3), familiar with concepts (2), and unfamiliar (1).

Questions were validated by a panel of experts before conducting a pilot test involving 10 college staff, who were not included in the study, followed by interviews with the respondents to ensure the validity of each item. Data collected from the pilot test were then tested for the internal consistency of the questionnaire by using SPSS, which revealed a reliability coefficient (Cronbach alpha) of 0.86. The questionnaire was then administered to the participants through a web link.

Descriptive statistics (frequencies) were completed for all items. The results were analyzed with the use of SPSS software version 20.0 (SPSS, Chicago, Illinois). We used a two-sample *t*-test between percent and *N* - 1' chi-squared test to compare the differences in distribution by calculating the 95% confidence interval and the *P*-value for

statistical significance. The statistical significance was set at $P < 0.05$ for all analyses.

Results

Out of the 45 nominated, 36 (80%) have successfully completed the questionnaire. The demographical features of the participants are shown in Table 1. The participating members represent most of the molecular diagnostics disciplines. At least 29 (80.6%) of our respondents have a doctorate degree. The average experience of all participants is 11.0 ± 6.7 years. While 55.6% of the respondents belong to laboratories of the health-care centers, 38.9% belong to universities, and 5.6% belong to research centers.

Table 1 Demographic Features of the Participants

Variables	Frequency
Position	
Professor of molecular medicine/biology, PhD	3
Associate professor in molecular microbiology, PhD	4
Associate professor in molecular biology/medicine, PhD	4
Associate professor in forensic biology, PhD	2
Assistant professor in molecular immunology, PhD	1
Scientist at the Medical Genomic Research Department	1
Consultant, molecular hemato-oncology, MD	2
Consultant, HLA molecular laboratory, MD	2
Scientist/researcher in molecular genetic/cytogenetic, MD	5
Consultant, newborn screening/biochemical genetics/metabolic laboratory, MD	3
Consultant molecular virology, MD	2
Laboratory manager, laboratory technologist, MSc	5
Molecular diagnostics laboratory supervisor, MSc	2
Workplace	
Health care center laboratory	20
University	14
Research center	2
Experience	
1–3 years	5
4–10 years	14
11–20 years	11
>20 years	6
Gender	
Male	27
Female	9
Total	36

Table 2 Currently Performed Molecular Methods (Yes) or Not (No) or Planned to Be Performed in the Next 5 Years (Planned) and the 95% Confidence Interval of the Used Methods

Method	Yes	Planned	No	95% CI	P
DNA/RNA isolation	86.1	11.1	2.8	64.7–91.5	< 0.0001
Conventional qualitative PCR	77.8	16.7	5.6	52.0–83.5	< 0.0001
Quantitative PCR	80.6	11.1	8.3	51.6–83.3	< 0.0001
Nested PCR	41.7	0.0	58.3	–6.15–37.2	0.1604
Multiplex PCR	75.0	16.7	8.3	45.7–79.2	< 0.0001
RT-PCR	72.2	11.1	16.7	33.3–70.4	< 0.0001
RFLP	50.0	0.0	50.0	–22.0–22.0	1.0000
MLPA	36.1	8.3	55.6	–13.8–29.3	0.4743
Other amplific tech (ie, bDNA amp)	41.7	8.3	50.0	–14.1–29.6	0.4812
Gel electrophoresis	69.4	16.7	13.9	33.5–70.3	< 0.0001
Southern blotting	36.1	11.1	52.8	–6.0–37.1	0.1575
FISH	61.1	0.0	38.9	–0.8–42.2	0.0612
Sanger sequencing	41.7	16.7	41.7	–22.0–22.0	1.0000
Pyrosequencing	22.2	0.0	77.8	33.1–70.4	< 0.0001
Next generation sequencing	30.6	25.0	44.4	–8.2–34.2	0.2271
Array (microarray, bead array)	44.4	22.2	33.3	–11.0–31.8	0.3370
DHPLC	27.8	8.3	63.9	13.1–54.2	0.0023
GCMS	33.3	0.0	66.7	10.2–51.9	0.0050
MALDI	27.8	16.7	55.6	5.0–46.9	0.0176
Primer design	41.7	13.9	44.4	–19.2–24.4	0.8137
Assay development	52.8	16.7	30.6	–0.5–42.0	0.0576
Assay verification/validation	83.3	8.3	8.3	54.7–85.3	< 0.0001

Abbreviations: RFLP, restriction fragment length polymorphism; DHPLC, denaturing high-performance liquid chromatography; MLPA, multiplex ligation-dependent probe amplification; FISH, fluorescent in situ hybridization; GCMS, gas chromatography-mass spectrometry; MALDI, matrix-assisted laser desorption ionization.

Participants reported that most of the 22 listed molecular techniques/procedures that included amplification techniques, enzymatic-based methods, electrophoretic-based techniques, solid phase-based hybridization or blotting techniques, microarrays, sequencing, DNA/RNA isolation, primer design, and assay development and verification, are commonly practiced or with few exceptions (Table 2). These techniques are commonly used or planned to be used within the next five years by more than 60% of the participating laboratories.

The least practiced techniques were nested PCR, multiplex ligation-dependent probe amplification (MLPA), Southern blotting, pyrosequencing, next-generation sequencing, gas chromatography-mass spectrometry (GCMS), denaturing high-performance liquid chromatography (DHPLC), and matrix-assisted laser desorption ionization (MALDI). Even these techniques are used on average by more than 30% of the surveyed laboratories. Overall, 62.4% have used or planned to use the mentioned techniques. Techniques that were not

Table 3 Expected Levels of the Graduate Competencies and the Score Rate Out of Four of the Various Molecular Techniques

Method	Expert %	Familiar with Skills and Concepts %	Familiar with Concepts %	Unfamiliar %	Score Out of 4
DNA/RNA isolation	77.8	16.7	0.0	5.6	3.7
Conventional qualitative PCR	66.7	22.2	8.3	2.7	3.5
Quantitative PCR	61.1	16.7	13.9	8.3	3.3
Nested PCR	11.1	38.9	33.3	16.7	2.4
Multiplex PCR	52.8	19.4	22.2	5.6	3.2
RT-PCR	58.3	25.0	11.1	5.6	3.4
RFLP	8.3	33.3	33.3	25.0	2.2
MLPA	11.1	22.2	50.0	16.7	2.3
Other amp tech (ie, bDNA amp)	8.3	16.7	44.4	30.6	2.0
Gel electrophoresis	69.4	25.0	0.00	5.6	3.6
Southern blotting	25.0	41.7	16.7	16.7	2.7
FISH	22.2	33.3	25.0	19.4	2.6
Sanger sequencing	33.3	16.7	38.9	11.1	2.7
Pyrosequencing	0.0	33.3	33.3	33.3	2.0
Next generation sequencing	22.2	25.0	33.3	19.4	2.5
Array (microarray, bead array)	19.4	44.4	25.0	11.1	2.7
DHPLC	16.7	11.1	50.0	22.2	2.2
GCMS	27.8	16.7	41.7	13.9	2.6
MALDI	16.7	8.3	41.7	33.3	2.1
Primer design	25.0	33.3	33.3	8.3	2.8
Assay development	27.8	33.3	30.6	8.33	2.8
Assay verification/validation	44.4	22.2	33.3	0.0	3.1

Abbreviations: RFLP, restriction fragment length polymorphism; DHPLC, denaturing high-performance liquid chromatography; MLPA, multiplex ligation-dependent probe amplification; FISH, fluorescent in situ hybridization; GCMS, gas chromatography-mass spectrometry; MALDI, matrix-assisted laser desorption ionization.

used by >50% of the respondents' sites included nested PCR, MLPA, GCMS, MALDI, and pyrosequencing. About 32.1% required expert skills for all listed techniques/procedures. Expert level of skills was requested for DNA/RNA isolation (77.8%) followed by gel electrophoresis (69.4%) and the various types of PCR except for nested PCR (Table 3).

The expert and familiar with skills and concepts responses were given by 57.4%, compared to 28.2% who suggested they only had familiarity with the concepts. Only 14.5% gave the unfamiliarity response.

In general, the overall score of skills expectations was 2.8 ± 0.5 out of four. The highest levels of the graduate skills were expected for the various types of PCR, gel electrophoresis, primer design, assay development, and verification.

The practice level for molecular techniques was in favor of a master's degree (53.8%) (Table 4). Most of the respondents agreed that nucleic acid isolation, electrophoresis, and PCR could be practiced by bachelor-level personnel. Other techniques that were highly recommended to be done by master's level personnel

Table 4 Level of Expertise the Expected Graduates of the Program Should Have

Level	B. Sc	M. Sc	95% CI	P
DNA/RNA isolation	77.8	22.2	33.1–70.4	< 0.0001
Qualitative PCR	80.6	19.4	39.1–74.8	< 0.0001
Quantitative PCR	58.3	41.7	–6.2–37.2	0.1604
Nested PCR	50.0	50.0	–22.0–22.0	1.0000
Multiplex PCR	38.9	61.1	–0.8–42.2	0.0612
RT-PCR	63.9	36.1	4.7–47.1	0.0192
RFLP	41.7	58.3	–6.2–37.2	0.1604
(MLPA)	27.8	72.2	21.5–61.3	0.0002
Other amplification techniques (ie, bDNA amp)	38.9	61.1	–0.8–42.2	0.0612
Gel electrophoresis	75.0	25.0	27.3–66.0	< 0.0001
Southern blotting	61.1	38.9	–0.8–42.2	0.0612
FISH	69.4	30.6	15.8–56.6	0.0011
Sanger sequencing	36.1	63.9	4.7–47.1	0.0192
Pyrosequencing	50.0	50.0	–22.0–22.0	1.0000
Next generation sequencing	25.0	75.0	27.3–65.9	< 0.0001
Array (microarray, bead array)	33.3	66.7	10.2–51.9	0.0050
DHPLC	30.6	69.4	15.8–56.6	0.0011
GCMS	25.0	75.0	27.3–65.9	< 0.0001
MALDI	22.2	77.8	33.1–70.4	< 0.0001
Primer design	47.2	52.8	–16.7–27.1	0.6394
Assay development	27.8	72.2	21.5–61.3	0.0002
Assay verification/validation	36.1	63.9	4.7–47.1	0.0192
Average	46.2	53.8	–14.8–29.0	0.5230

Notes: Two-sample *t*-test between percent. N = 1' chi-squared test.

Abbreviations: RFLP, restriction fragment length polymorphism; DHPLC, denaturing high-performance liquid chromatography; MLPA, multiplex ligation-dependent probe amplification; FISH, fluorescent in situ hybridization; GCMS, gas chromatography-mass spectrometry; MALDI, matrix-assisted laser desorption ionization.

Table 5 Rate the Following Issues of Clinical Quality Control and Management in Terms of How Expert Recent Graduates of a Master's Degree Program in Molecular Diagnostics Should Be

	Expert	Familiar with Skills and Concepts	Familiar with Concepts	Unfamiliar	Score
Quality assurance	41.7	58.3	0	0	3.4
Proficiency testing	50.0	50.00	0	0	4.0
Regulatory/accreditation requirements	30.6	69.4	0	0	3.3
Assay validation	69.4	30.6	0	0	3.7
Laboratory safety	94.4	5.6	0	0	3.9

Table 6 Program Chart of Didactic and Practical Training

Phase	Didactic and Practical	Credits
Semester 1	<ul style="list-style-type: none"> • Biochemistry of Nucleic Acids and Proteins • Molecular Genetics • Basic Molecular Biology • Research Methods and • Biostatistics and Bioinformatics 	15
Semester 2	<ul style="list-style-type: none"> • Molecular Diagnostics I: Microorganisms • Molecular Diagnostics II: The Molecular Basis of Inherited Diseases and neonatal screening • Molecular Diagnostics III: Molecular Oncology and Hematology • Molecular Diagnostics IV: Human Identification, DNA-based tissue typing, and pharmacogenomics • Quality Assurance in Molecular Diagnostics Molecular Laboratory 	16
Semester 3 and 4	<ul style="list-style-type: none"> • Research Project in Molecular Diagnostics 	8
Total		39

were MALDI, next generation sequencing, DHPLC, MLPA, and assay development.

All participants have agreed that a master's graduate should be an expert or at least familiar with skills and concepts of laboratory safety, quality assurance, accreditation, and performance and procedural documentation to maintain compliance with governing agencies (Table 5).

The level of skills expectation is so high for the specific managerial and quality activities with an overall of 3.7 ± 0.3 out of four indicating that there should be special emphasis on these topics in the proposed program (Table 6).

Discussion

The curriculum design process involves several steps. The first step is to gather information about what the learner needs, design the blueprint, and build the content before the final evaluation. The common methods to gather information on the standard requirements of the professional practice and on its anticipated future directions are through surveys and interviews with professional practitioners and employers.^{25–27} Learning outcomes must be properly sequenced and include necessary content and activities to enable students to achieve competencies in each major discipline. Finally, evaluation methods must measure the course learning outcomes and support program competencies. The evaluation methods must be performed often in order to be used as a reliable parameter of the effectiveness of the course design and teaching strategies. Master's level courses are taken with a higher cognitive level of

learning outcomes to improve the learner's critical thinking and problem-solving skills with assignments in each didactic course and clinical rotation.

The ad hoc committee of the Academy of Clinical Laboratory Physicians and Scientists (ACLPS) has suggested several learning objectives pertinent to molecular diagnostics in laboratory medicine curriculum for medical students.²⁸ Suggested objectives for molecular diagnostics are that the graduating student should explain the general principles of molecular diagnostics testing in the screening, diagnosis, and/or monitoring of infectious, genetic, and oncologic diseases, and describe the place of pharmacogenetic testing in clinical care. Secondly, they should describe the legal, ethical, and social implications of genetic testing. Finally, they should compare and contrast genetic testing techniques, including amplification, sequencing-based tests, cytogenetic, fluorescence in situ hybridization, comparative genomic hybridization, and other common methodologies, and the limitations of each, as well as sources of false-positive and false-negative genetic tests; and understand quantitative polymerase chain reaction testing.

We designed the master program on molecular diagnostics based on the information gathered from the literature, the feedback of our stakeholders, and following the guidelines of the NACCLS and the NAACLS Academy of Clinical Laboratory Physicians and Scientists.^{16,29} The two major messages from the stakeholders are that both cognitive and psychomotor skills of the mentioned molecular techniques are required for the program and there is a need to include extensive laboratory training during the courses.

Our curriculum was developed after gathering information from the literature and following the National Accrediting Agency for Clinical Laboratory Sciences (NAACLS) guidelines and collecting feedback from consultants of other universities in the area and the stakeholders performing molecular diagnostics at their laboratories. We have compiled all these data for developing a molecular diagnostics curriculum for a master's level of education.

According to the NAACLS the unique standards for the curriculum of molecular diagnostic must address pre-analytical, analytical, and post-analytical components of diagnostic molecular laboratory services covering diagnostic molecular tests used to detect or diagnose acquired and genetic diseases. This includes principles, methods, and performance of assays, and problem-solving. Additionally, troubleshooting techniques, interpretation and evaluation of methods and results, statistical evaluation of data, quality assurance/improvement, and continuous assessment of laboratory services must be included. In addition, principles, methodologies, and applications of molecular microbiology, molecular pathology (hematology/oncology), and molecular genetics must be present. Other common laboratory practices such as safety, professional conduct and development, communication skills, administration, supervision, and quality management applied to diagnostic molecular science were also included.

Based on the current findings and aligned with the NAACLS and ACLPS guidelines the curriculum for this program was developed. This program covers topics on the principles and techniques of molecular biology and clinical applications of molecular testing in order to acquire knowledge of the molecular basis of health and disease. It includes molecular microbiology and infectious diseases, genetic testing in inherited diseases and their clinical applications, molecular oncology, hematopathology, newborn screening, pharmacogenomics, precision medicine, tissue typing, and others.

The program introduces the basics of molecular biology and genetics, principles of nucleic acids, including DNA and RNA extraction and detection, electrophoresis and blotting, and principles of several direct and amplified nucleic acid test methods, and clinical applications are discussed. Furthermore, the principles of automated DNA sequencing and various methods of genotyping and mutation analysis were also included. In addition, we included human identity by DNA typing; principles and

applications of quantitative-PCR; basic concepts of molecular cytogenetics; in situ hybridization techniques; principles and applications of flow cytometry; emerging technologies (epigenetics, transcriptional profiling, next-generation sequencing, stem cells, gene editing using CRISPR), pharmacogenomics, and laboratory management issues of molecular testing.

For the fulfillment of the master's degree, candidates will need to submit a project report on a clinical and/or laboratory project, which should be conducted over a period of one year either within the candidate's own care center or practice or in a host laboratory. The candidate is expected to design a research project which should reflect an application of the knowledge acquired during the first year of the program. The choice of topic for the study should be discussed in advance with an identified supervisor who will give guidance on the writing of the project report.

The main purpose of assessment is to test how well the student has learnt and mastered the course objectives, to validate the efficacy of the teaching methodology and strategies, and to evaluate the entire content of the course.

The overall assessment for each course consists of continuous assessment and end-of-course examinations and consists of different performances: quality and completeness of the work done, active participation in the scientific discussion, and attendance. Continuous assessment is carried out on work completed and marked during the semester. A predetermined portion of these marks is secured from work carried out by the student under formal conditions (eg, study unit tests, classroom written and oral tests, practical tests/exam, mid-semester examination). Other marks, allocated to continuous assessment, are obtained from work carried out under non-formal circumstances (eg, homework exercises, practical and clinical work). All such marks are weighted and combined to yield the overall continuous assessment mark, which must fall within the range, 40–60%, of the overall mark given to the course.

Our study has determined a real need for master graduates who can find employment in numerous types of settings such as hospitals, public health departments, pharmaceutical companies, research institutions, and forensic laboratories. The knowledge and skills gained on this program also provide a solid grounding for PhD studies.

The only limitation of this study is that it is questionnaire-based and may be a subject of recall bias.

Disclosure

The author reports no conflicts of interest in this work.

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