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Development of Concept Mastery Tests Polymerase Chain Reaction in Molecular Biology

Evi Suryanti[1,2,a)], Any Fitriani[1,3], Sri Redjeki[1], and Riandi[1,3]

[1]*School of Postgraduate Studies, Indonesia University of Education*
Jl. Dr. Setiabudi No. 229, Bandung 40154, Indonesia
[2]*Program of Biology Education, Islamic University of Riau*
Jl. Kaharuddin Nasution No. 113, Pekanbaru 28284, Indonesia
[3]*Department of Biology Education, Indonesia University of Education*
Jl. Dr. Setiabudi No. 229, Bandung 40154, Indonesia

[a)]Corresponding author: evisuryanti97@student.upi.edu

Abstract. This study aims to develop a polymerase Chain Reaction (PCR) concept mastery test that can be used in biology learning, especially in the fields of molecular biology, biotechnology, and genetics. Approach of Research & Development of ADDIE (Analysis, Design, Development, Implementation and Evaluation) model is used in this research. The concept mastery test consists of 21 multiple choice questions with four answer choices developed based on the theoretical and techniques content on PCR. An analysis of the test obtained data that 12 items are valid and 9 items are invalid; reliability level is 0.5 (good enough); 3 items are easy, 12 items are medium, and 6 items are difficult questions; 10 items have good criteria for discriminating power, 5 items are good enough, 4 items are poor, and 2 items are very ugly. It was concluded that the the Polymerase Chain Reaction (PCR) mastering test is not yet valid and reliable.

INTRODUCTION

Molecular biology is a branch of science that discusses the relationship between the structure and function of biological molecules and the contribution of those relationships to the implementation and control of various biochemical processes. The major studies of molecular biology are biological macromolecules, especially nucleic acids (DNA and RNA), as well as processes for the maintenance, transmission, and expression of biological information that include replication, transcription, and translation. More concisely, it can be stated that molecular biology exposes the molecular basis of each biological phenomenon [1]. One of the important basic techniques to understood in molecular biology is polymerase chain reaction (PCR) techniques.

PCR is a method of multiplying specific segments of DNA quickly and specifically [2]; one of the most sophisticated techniques that continues to evolve in the area of recombinant DNA research to amplify the short DNA segments of the genome by in vitro within a few hours [3]. The multiplication of DNA segments by PCR technique differs from DNA duplication during cloning and propagation in a host cell as it takes place in vitro. PCR has a major impact on most areas of molecular cloning and genetics. In addition to its use in molecular cloning strategies, PCR is also used in gene expression analysis, forensic analysis for minimal DNA samples isolated from crime scene, and diagnostic testing for genetic diseases [3].

The magnitude of the benefits of PCR techniques in human life became the basis of the importance of this technique is understood by prospective biology teachers. Strong mastery of the PCR concept becomes one of the capital for prospective biology teachers who will teach biotechnology concepts in high school so that biotechnology learning becomes meaningful for learners. To measure the understanding and mastery of student biology teacher candidate on the concept of PCR can be done with test techniques. The test is a set of questions or statements relevant to the test objectives that have been planned by the test taker [4]. The test is one of the official means of gathering information because it has limits [5]. In the context of the evaluation process conducted in educational institutions, especially in the classroom, the test has a dual function that is to measure the achievement of learners and to measure the success of the learning program. Judging from the authors, the tests were distinguished on teacher-made tests and standardized tests. The purpose of this study

is to develop a test instrument used to measure the mastery of student biology teacher candidate on the concept of PCR, and analyze the items to know the quality of the test.

METHOD

Test Instrument Development Procedure

This research uses approach of Research & Development (Research and Development) of ADDIE model which consists of five phases: analysis, design, development, implementation and evaluation. The ADDIE model is used because it is one of the most widely used models as a guide in designing effective teaching, it can be used in any environment either online or face-to-face. Each phase in the ADDIE model is interconnected and interacts with each other [6]. The evaluation phase in the ADDIE process provides feedback that enhances the continuing professional development (CPD) program [7].

Analysis Phase: Reviewing the molecular biology syllabus or semester plans (RPS) from several public and private universities, core competencies and basic competence of Biology/IPA subjects at the secondary school level, and various learning resources related to molecular biology. Further set goals and objectives of the development of test instruments.

Design Phase: formulate performance indicators to be measured and form tests to be developed.

Development Phase: making the initial design or form of the test instrument. The design of this test instrument is validated by promotor team of experts in molecular biology content and pedagogy.

Implementation Phase: conducting field trials of validated tests to 45 students of IPA-Biology Tadris Program at IAIN Sheikh Nurjati Cirebon who contracted the course of molecular biology.

Evaluation Phase: analyzing test instruments statistically includes analysis of validity, reliability, difficulty level, and the discrimination power of items.

Statistical Analysis Procedure

Validity of the item. To know the validity of item is used biserial correlation formula (r_{pbi}) [5]. Furthermore t_{count} value is determined by t test. Decision-making is based on criteria: if the t is positive, and $t_{count} > t_{table}$, then the item is valid, and if t_{count} is negative, and $t_{count} < t_{table}$, the item is invalid.

Reliability of test instruments. The reliability coefficient was obtained using the KR-21 formula because the item has a multiple choice of four answers [4]. Center for Research, Curriculum and Instruction states that the reliability of the instrument is said to be very good if the reliability coefficient ranges from 0.70 or 0.80 [8].

Difficulty of item. Difficulty level of item (P) is the percentage of students who correctly answer (N1) divided by the total number of answers (N). Thus, a low P value indicates a difficult question [9]. The difficulty level of the item is expressed by an index of difficulty whose value ranges from 0 to 1 [5]. The criteria used to interpret the difficulty index are as follows:

Table 1. Criteria for an index of difficulty

Difficulty index (P)	Criteria
0,00 - 0,30	Difficult
0,31 - 0,70	Moderate
0,71 - 1,00	Easy

The discrimination power of the item. There are different ways of determining the discrimination power of items between small groups (less than 100 students) with large groups (more than 100 students). For small groups, all students participating in the test were divided equally between 50% of the upper group and 50% of the lower group [5]. In this study, the students numbered 45 people, so divided into two groups as large as to analyze the discrimination power of items. The magnitude of the discrimination power is expressed by the discriminating index (D) ranging from 0 to 1. The high D value indicates that only the smart students answer the question correctly [9]. Interpretation for index discrimination (D) follows the following criteria:

Table 2. Criteria for discrimination index

Discrimination index (D)	Criteria
0,00	Very ugly
0,01 - 0,20	Poor
0,21 - 0,40	Good enough
0,41 - 0,70	Good
0,71 - 1,00	Excellent

RESULTS AND DISCUSSION

Process and Development Results of Test Instruments

The analysis phase in this research begins by collecting and reviewing molecular biology syllabus from several Biology Education Study Program which has molecular biology subject. The results of the study of the syllabus obtained data that the concept of PCR is one of the concepts discussed in the lectures of molecular biology. This shows that the concept of PCR is an important concept to be supplied to prospective biology teachers.

The design phase begins with determining the purpose of the development of the test, limiting the materials/materials to be tested, formulating the achievement indicators in learning the concept of PCR, determining the appropriate form of test type, and create a lattice table about test. Related to this, [10] describes the assessment process for the type of test including: determining the purpose of the assessment, determining the competence tested, formulating the competence support materials, and determining the appropriate type of test (written, oral, performance). According to Barnard [4] the purpose of the tests prepared in the evaluation process there are two kinds of categories, namely the bureaucracy and the professional categories. The purpose of the test in this study included the professional category as a research effort to extract information from the students related to whether or not there is improvement of mastery of PCR concept, whether the learning process that can fulfill the achievement indicators, and whether the learning process pleases the students.

The achievement indicators formulated in the PCR learning are four, namely a) Students are able to determine PCR steps, b) Students are able to identify the equipment used in the PCR process, c) Students are able to determine the materials required in the PCR process, and d) Students are able to analyze the process and results of PCR with the help of virtual laboratory media. This achievement indicators are based on a review of the various literatures describing the theory and techniques of PCR and the role of PCR in biotechnology. The development of biotechnology is supported by the role of PCR techniques in expanding foreign genes of interest.

The type of test instrument developed is multiple choice test with four alternative answer options. In theory, there are two types of tests: norm-referenced test and criterion-referenced mastery test [4]. This developed test refers to the criterion-referenced mastery test used to measure students' mastery of the concept of PCR based on certain predetermined criteria without comparing with other student mastery. Multiple-choice tests are selected because this type of test has all the requirements as a good test, i.e. in terms of objective, reliability, and the ability to distinguish between smart and low-ability students [4]. This type of test can be used to measure students' ability to know facts related to PCR and evaluate the application of expository methods, focus group discussions, and presentations with the help of virtual laboratory media in the PCR learning process.

Development phase followed by writing the items according to the lattice table about test that has been created so as to form a test instrument mastery of the concept of PCR containing 21 items. The process of developing this test instrument is guided by 3 experts in molecular biology content and pedagogy. Once complete, the supervisor recommends that the test instrument be implemented to be able to know the quality.

The implementation phase is the stage of piloting the PCR test instrument to 45 students of IPA-Biology Tadris Program at IAIN Syekh Nurjati Cirebon, Jawa Barat who contracts the course of molecular biology. The learning of PCR concept is done by expository, focus group discussion, and presentation method with virtual laboratory-assisted media which can be downloaded from internet.

Evaluation phase is an important step that must be done to know the quality of tests that have been made. For this purpose, an analysis of the items has been analyzed.

Validity

One of the factors that determine a test is a high degree of validity. The meaning of validity in the opinion of some experts [10] is: the accuracy of the interpretation of measurement results (Linn and Gronlund), the significance of the test scores (Cohen, et al.), the interpretation or meaning and the use of the student achievement (Nitko), and the integration of evaluative considerations degree of empirical information that bases theoretical thinking that supports accuracy and conclusions based on test scores (Messick). Validation aims to determine the suitability between the items with the indicator of the achievement set. An analysis of the validity of an instrument is required to know that the instrument used can measure what it wants to measure [4]. The results of the validity analysis are summarized in Table 3 below:

Table 3. Analysis of the item validity

Criteria	Item
Valid	2, 3, 4, 6, 7,8, 9, 12, 14, 15, 16, 19
Invalid	1, 5, 10, 11, 13, 17, 18, 20, 21

The analysis results show 12 valid items and 9 items are invalid. In general it can be said that these test instruments have not met the criteria of good tests. According to Linn and Gronlund [10] a good test should meet three characteristics, namely validity, reliability, and reusability. Factors that cause low validity because students have difficulty answering questions about PCR techniques related to the absence of hands-on activities in the form of PCR practice conducted by students. The importance of the laboratory in science learning can be understood in relation to the three goals of the laboratory in the reform curriculum, which is to motivate students, form active learning and illustrate the scientific method. Students can develop ideas that reflect laboratory experience and build knowledge based on these ideas [11].

The items 5, 11, 17 (Table 3) are items for the second achievement indicator 'Students are able to identify the equipment used in the PCR process' and items 10, 13, 20 are items for the third achievement indicator, ie 'Students are able to determine the materials needed in the PCR process'. Both of these achievement indicators require a hands-on activity to enable students to identify equipment and materials used in the PCR process, so that students can answer the test questions correctly.

Another factor that causes the low validity of this test is the number of small items. The validity of a test may be influenced by both internal and external factors of the test as well as the participant factor [4]. Limitations in this study is not conducted interviews of students test participants so it is not known for sure the cause of the low validity of the test in terms of factors test participants.

Reliability

Reliability analysis results obtained reliability coefficient of 0.5 (good enough). High reliability coefficient indicates high instrument reliability; conversely, reliability is low if the reliability coefficient is low. The reliability coefficient rates typically range from -1 to +1 [4]. The low reliability of the test instrument is due to the small number of question items and the number of items that are not valid, because of the validity and reliability are interconnected. Reliability is required to support the formation of validity, so a valid test must be reliable [5]. Center for Research, Curriculum and Instruction states that the reliability of the instrument is said to be very good if the reliability coefficient ranges from 0.70 or 0.80 [8].

Cooper and Schindler [12] argue that reliability can be increased by increasing the number of items. This opinion is supported by research [13] which obtained a reliability coefficient of 0.85 in the development of 43 items (biology: 15, chemistry: 13, physics: 15) for the final energy assessment assessment instrument for grade 6, 8 and 10 students of 752 students.

Level of Difficulty

A good question is not too easy or too difficult. An easy question does not stimulate the student to solve it. On the contrary, the question is too difficult to make students desperate and not eager to answer [5]. The results of the analysis on the difficulty level of the item can be seen in Table 4 below:

Table 4. Analysis of difficulty index

Criteria	Item
Easy	1, 11, 12
Moderate	2, 4, 5, 6, 7, 8, 9, 14, 15, 18, 19, 21
Difficult	3, 10, 13, 16, 17, 20

There are 3 easy items, 12 moderate items, and 6 difficult items. Judging from the difficulties distribution of the item, the test instrument can be said to be a good test because the number of moderate items more than the number of items is easy and difficult. The items that are considered good are those that have a difficult index of 0.30-0.70 (moderate criterion) [5]. However, the level of difficulty for criterion-referenced mastery test is not so much based on the item ability to distinguish between high and low students in answering questions in a class. The difficulty of each question in a criterion test is principally determined by the learning outcomes to be measured. That is, if the task specified in the learning result is easy then the item made is also easy. Thus, in tests referring to the criterion-referenced mastery test there is no attempt to change the difficulty level of the matter without looking at the type of predetermined task [4].

Discrimination Power

The discrimination power states the ability of the item to distinguish between high and low-ability students. Distribution of items based on the results of analysis of power discrimination can be seen in Table 5 below:

Table 5. Analysis of discrimination power of item

Criteria	Items
Good	2, 4, 5, 7, 8, 9, 14, 15, 16, 19
Good enough	3, 6, 10, 12, 21
Poor	1, 13, 17, 20
Very ugly	11, 18

The six items have an ugly discrimination because they can not distinguish a group of highly and low-ability students. A good item is an item that has a discriminating index of 0.41-0.70 (good criterion) [5]. If associated with validity, the six items are invalid item. If associated with the level of difficulty, the items are mostly an easy and difficult criteria except for the number of 18 are moderate criteria. So the good items with the level of difficulty 0.31-0.70 (moderate criteria) does not mean have a good discrimination also. A good item with a difficulty level of 0.31-0.70 (moderate criterion) does not mean that the item has a good discrimination as well. The items with a value of $P = 0.50$ allow to have the highest discrimination power ($D = 1.00$) [5]. In the meantime, 5 items with good enough of discrimination power can still be used or reviewed and corrected so that the power of discrimination may increase. The value of the discrimination item is also useful for understanding how the performance of the item is related to the overall performance of the test instrument [9].

The good of a test instrument is not always indicated by the results of the item analysis. Another factor that also determines the quality of a test is the ability of the student in mastering the concepts he studied. The better the learners master the learned concepts, the greater the chance to answer the question correctly [10]. Teachers should plan learning strategies to teach a concept after choosing the concept to learn. For that, the teacher not only mastered the knowledge of the field of study to be taught, but also the various approaches and methods of learning as well as various learning theories to guide teachers in applying the approach and method chosen [14].

The use of the ADDIE model in this study provides convenience in the development process of PCR test instruments. This model is systematic because it guides step by step test instrument development process. The ADDIE model has been widely used for being effective and systematic [15], easy to use and easy to apply to curricula that teach knowledge, skills or attitudes [16].

CONCLUSION

This PCR concept mastering test has 9 items that are invalid, the level of reliability is good enough, the distribution of difficulty level is good, and 6 items that the discrimination power is poor. It was concluded that the PCR test instrument is not yet valid and reliable. It is recommended that these test instruments be improved from the content and construct aspects in order to improve their validity and reliability. in addition it is also recommended to use learning strategies that can improve the mastery of the pcr concept, such as inquiry learning, hands-on activity of wet laboratory method that is interfaced with virtual laboratory.

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REFERENCES

1. H. Susanto, *Bahan Ajar Biologi Molekuler*, (Fakultas Biologi, Universitas Jenderal Soedirman: Semarang, 2012), p. 1.
2. N. A. Campbell, J. B. Reece, L. A. Urry, M. L. Cain, S. A. Wasserman, P. V. Minorsky, and R. B. Jackson, *Biologi* (Erlangga, Jakarta, 2010), p. 428.
3. L. A. Allison, *Fundamental Molecular Biology*, (Blackwell Publishing: USA, 2007), pp. 206, 207, 589.
4. Sukardi, *Evaluasi Pendidikan: Prinsip & Operasionalnya*, Edisi Pertama, (Bumi Aksara: Jakarta, 2011), pp. 31, 38, 43, 49, 88, 125, 134, 139.
5. S. Arikunto, *Dasar-dasar Evaluasi Pendidikan*, Edisi Kedua, (Bumi Aksara: Jakarta, 2013), pp. 47, 93, 101, 225, 227, 232, 233.
6. N. Aldoobie, *American International Journal of Contemporary Research*, **5** (6), 68-72, (2015).
7. R. K. Almomen, D. Kaufman, H. Alotaibi, N. A. Al-Rowais, M. Albeik, and S.M. Albattal, *International Journal of Clinical Medicine*, **7**, 538-546, (2016).
8. Center for Research, Curriculum and Instruction, *Validity, Reliability and Item Analysis,* (2016).
9. J. Shi, W. B. Wood, J. M. Martin, N. A. Guild, Q. Vicens, and J. K. Knight, *CBE-Life Sciences Education*, **9**, 453–461, (2010).
10. Safari, *Penulisan Butir Soal Berdasarkan Penilaian Berbasis Kompetensi*, (Asosiasi Pengawas Sekolah Indonesia, Departemen Pendidikan Nasional: Jakarta, 2005), pp. 11-12.
11. M. C. Linn, *The Elementary School Journal*, **97** (4), 401-417, (1997).
12. L. Cohen, L. Manion, and K. Morrison, *Research Methods in Education*, Sixth Edition, (Taylor & Francis e-Library, 2007), p. 506.
13. S. T. Opitz, K. Neumann, S. Bernholt, and U. Harms, *Eurasia Journal of Mathematics Science and Technology Education*, **13** (7), 3019-3042, (2017).
14. R. W. Dahar, *Teori-teori Belajar & Pembelajaran*, (Erlangga: Jakarta, 2011).
15. S. Danks, *ASQ Primary and Secondary Education Brief*, **4** (5), (2011).
16. L. Cheung, *Journal of Biomedical Education*, (2016).