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Spermatozoa Morphology Examination Using Lenshooke™ SQA X1 Pro Compared with Manual Method

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Abstract

According to World Health Organization data, 30-40% of infertility is caused by male factors. The morphology of normal spermatozoa is an indicator of male fertility, and it is known by manual or automatic sperm analysis. Lenshooke™ SQA X1 PRO automatic equipment comes along with the development of laboratory equipment automation technology. The working principle of this tool is by shining light on the object of examination, then the camera with high resolution, with the facility of an optical lens will take a picture of the object. The database recorded by the camera is analyzed by the algorithm. The research objective was to test the suitability of the Lenshooke™ SQA X1 PRO automatic tool with manual method as the Gold Standard. Subjects in this study were patients who carried out semen analysis tests at the Clinical Pathology Laboratory of RSIA "Restu Ibu" Sragen from June to August 2020. The examination method used an automatic method with the Lenshooke™ SQA X1 PRO tool and a manual method with Papanicolaou staining. The results of the study, conformity test with WHO 2010 normal standards, automatic methods reached 94.4% compared to manual methods. The next statistical test was with standard mean, normal sperm morphology data had a significance of 0.001, abnormal sperm head data had a significance value of 0.956 and abnormal sperm tail data had a significance value of 0.339. The Lenshooke™ SQA XI PRO device based on automatic technology can be used in laboratory services for sperm analysis in addition to manual methods. Suggestions for using the Lenshooke™ XI PRO automatic tools are still accompanied by the manual method.

Keywords

Automatic, Microscope, Morphology, Spermatozoa.

INTRODUCTION

The morphology of spermatozoa refers to the whole form of spermatozoa cells consisting of the head, middle and tail (1). Spermatozoa cells form in the seminal tubules that are in the testes. This tubule contains a complex series of cells, namely the development or division of cells from the germinal to the formation of spermatozoa or male gametes (2). About 300 million spermatozoa carry out the process of spermatogenesis mean in one day there are approximately 300 million spermatozoa newly manufactured (3).

According to data from the World Health Organization (WHO), 30 – 40% of infertility is caused by male factors, hence it is important to evaluate fertility of men as part of routine check. The basic test for infertility by performing semen analysis is the most commonly used diagnostic option. The result of semen analysis of 25% of infertile men was asthenozoospermia (abnormality of spermatozoa movement). The rest are disturbances in number (oligozoospermia) and morphology (teratozoospermia) or a combination of the three (4).

Semen analysis includes macroscopic and microscopic examinations. Microscopic analysis of normal semen results from parameters of concentration, motility and morphology to determine fertility in men. Normal spermatozoa cell morphology as a clinical tool for male fertility (5). Morphological abnormalities can make it difficult for spermatozoa to fertilize an egg (6). Spermatozoa show tremendous variability in size and shape. There is a correlation between the morphology of spermatozoa and reaching the female reproductive tract during the fertilization process (7).

Analysis of semen in manual method is the gold standard (WHO), which is widely used in laboratory of Clinical Pathology. This method has the advantage and disadvantage. Some of the findings in the field for the shortcomings of the manual method of semen analysis include that there are still differences in the interpretation of the results of inter laboratory examinations. The analysis process takes a relatively long time between 30 to 60 minutes, and the equipment used does not have the same standard (4).

The morphological examination of spermatozoa using an automatic method is present amidst the need for semen analysis. The development of accessible, fast and standard methods for semen analysis is urgently needed. The automated method provides a solution for semen analysis checks with fast results and good quality control standards (4). Semen analysis using automated with computer-based method to cover the shortage of existing shortcomings in the semen analysis manual methods (8).

The basis for the authors' consideration as Medical Laboratory Technologist Experts

in conducting research entitled Spermatozoa Morphological Examination Using the Lenshooke™ SQA X1 PRO Tool Compared to the Manual Method is because the authors still find that there are differences in the interpretation of semen analysis results between one laboratory and another. Some literature also states that manual semen analysis is a simple and inexpensive test, but has high variability and is very subjective (9).

Automatic method selections for spemartozoa analysis may use the Lenshooke™ SQA X1 PRO. This tool consists of software and hardware and the technical analysis works automatically (10). This is very practical and simple tool, which has four key parameters for evaluating male fertility, namely concentration, morphological, motility and pH. Lenshooke™ SQA X1 PRO yields very fast examination results which only take 3 to 5 minutes to get all the test results. Good quality control would be giving results in a accurate and reliable (11).

As a comparison, the researchers conducted a manual morphological examination of spermatozoa using Papanicolaou stain because this is one of the WHO Gold Standards for spermatozoa morphological staining (12). The aim of this study was to evaluate the spermatozoa morphology using Lenshooke™ SQA X1 Pro compared with manual method in order to provides reliable results.

MATERIALS AND METHODS

Morphology of spermatozoa were grouped into normal and abnormal morphology (5). Sampling was performed by using a consecutive sampling, that is sampling technique to assign subjects that met the study criteria. Samples were taken from patients who visited the Clinical Pathology Laboratory of Restu Ibu Hospital, Sragen, Indonesia from June 2020 to August 2020. The number of samples in this study was 48 patients, which are consist of the 36 patients who met the criteria for the study.

The inclusion criteria in this study were men in reproductive age between 20 to 45 years old and with spermatozoa concentration in excess of 2 million/mL. Exclusion criteria were semen samples with blood mixture, abstinence more than 7 days, liquefaction more than 60 minutes, increasing number of leukocyte in semen, and the number of immature spermatozoa cells.

Sperm Fluid Release

The release of sperm fluid for good results is by masturbating without using tools, such as gels, detergents and others. Sampling through sexual intercourse is not recommended. If circumstances compel sampling by sexual intercourse then use condoms and lubricants that are non-toxic and fertility-friendly if necessary. Collect complete sperm fluid, especially the first fraction rich in sperm.

Sample Handling

Prepare two sample pots, A and B, each with a patient identification label. The sample is accommodated in a clean, dry and wide-mouthed sample pot. The research sample for one identity is divided into two in the sample pot A and the sample pot B. Sample pot A for inspection of the Lenshoke SQA X1 PRO automatic method tool. Sample pot B for manual method examination with Papanicolaou stain. Each sample pot is done at the same time using two different inspection methods. To maintain the quality of the sample in the specimen pot, the temperature is kept around 20 – 37°C.

Manual Method

In the manual method, Papanicolau dye was used and considered that this dye is one of the dyes recommended by WHO. Staining Papanicolaou gives the results of the examination both for the morphology of spermatozoa and other cells. Papanicolaou staining has been proven and recommended by the WHO (12). Polychromatic staining is considered a very reliable staining technique. Factors that affect the coloring in addition to the use of dye solution, the time of painting, the duration of immersion, rinsing and immersion currents follow the standards that have been set. Figure 1 shows slides stained using the Papanicolaou procedure. This stain can be permanently installed and stored for use as internal quality control (13).

The principle of the staining of Papanicolau The Harirs's haematokxylin dye stains the cell nucleus blue, Orange G and EA 50 alcohol-based green coloring will work to color the cytoplasma. Ethanol 50% 80% 95% 100% for fixation and make cells become dehydrated and ethanol acid removes dyes undesirable but still attached especially to the cytoplasmic area. Water rehydrates cells (13). Procedure for manual method shows in Figure 1.

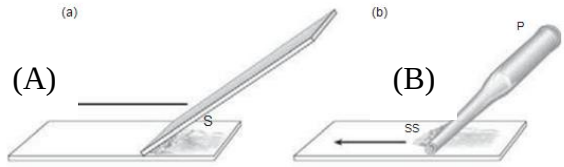

Figure 1. Preparation; 5-20 µL of semen is dripped on the object glass. (A) Move the slide to another object to make an erase. (B) Dry in the air 5-15 minutes (WHO, 2010).

Papanicolaou coloring

Soak the dried slides sequentially on ethanol 80% for 30 seconds, continue to ethanol 50% 30 seconds and soak in pure water for 30 second. Put into Harris hematoxylin stain for 4 minutes, then into pure water 30 seconds, put into ethanol acid for 8 seconds, flux with cold tap water for 5 minutes, and then into alcohol 50% for seconds, and 80% for 30 seconds, then dip

into ethanol 95% for 15 minutes. Stain with G-6 orange dye for 1 minute, and dip repeatedly into ethanol 95% for three times with 30 seconds each. Stain with EA-50 green dye for 1 minute, and then rinse with ethanol 95% two times with 30 second each. Final clearing rinse with ethanol 100% two times for 15 second each.

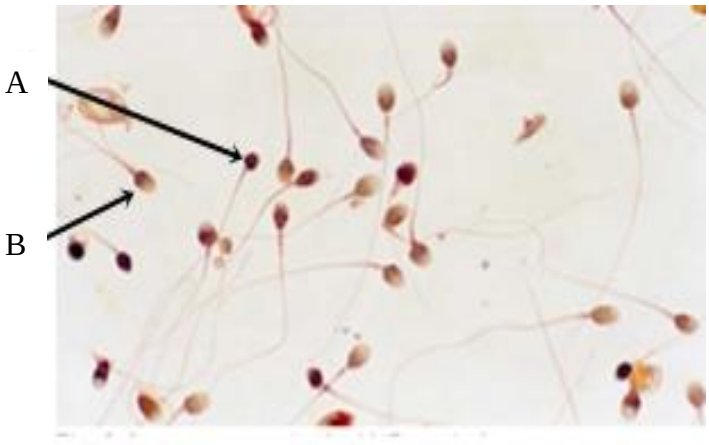

Figure 2. Morphology of spermatozoa with Papanicolau dye. (A) showing spermatozoa with amorphous head with thickened midpiece. (B) round head (14).

Result Reading

Morphology reading is the one semen analysis parameter in medical labotatory.The slides then read on a microscope with at least two technical officers. Using 1,000 times magnification assisted with immersion oil, observe the morphology of normal and abnormal spermatozoa. Report the percentage of observations of spermatozoa morphology as a result of the study (Figure 2).

The morphology of spermatozoa includes the assessment of the head, neck, middle and tail. The normal form of spermatozoa is a tadpole which consists of a blunt head in which there is a nucleus, and has a tail that contains an apparatus for moving. Morphologically abnormal spermatozoa are categorized into subgroups according to defects in the head, neck, midsection and tail (2). Several recent studies have demonstrated the importance of assessing the morphology of abnormal spermatozoa more carefully to establish the diagnosis of infertility (13).

Head Normal

The head of the sperm cell is oval with a size of 3 – 5 µm, there is a cell nucleus (nucleus) containing genetic information in the form of DNA in it. This genetic information will meet the genetic information from the egg and will determine whether the fetus is male or female. In the head of the spermatozoa, there are also enzymes, such as the hyaluronidase enzyme, which functions to penetrate the corona layer above the ovum, and protease enzymes (18).

Neck Normal

Neck is the area just behind the head that contains the centrioles. The middle part contains mitochondria arranged in a spiral, which contains energy (ATP) as an energy source for spermatozoa, for locomotion to the site of fertilization and for spermatozoa metabolism (3).

Tail

The tail of the spermatozoa is long with a size of 50 µm divided into the neck, the main/middle and the end (19). The main part is the longest part of the tail, and the end is the pointed end of the tail. The tail of the spermatozoa is in the form of flagella as a means of locomotion in the form of a long cytoskeleton that functions to propel the spermatozoa forward, at a speed of 30 inches/hour (18).

Abnormal

Abnormal is an abnormal form of spermatozoa. Morphologically abnormal spermatozoa are categorized into subgroups according to defects in the head, neck, midsection and tail (1). Several recent studies have demonstrated the importance of assessing the morphology of abnormal spermatozoa more carefully to establish the diagnosis of infertility (13). Term the results of semen analysis used to describe the morphological abnormalities of spermatozoa shows in Figure 3..

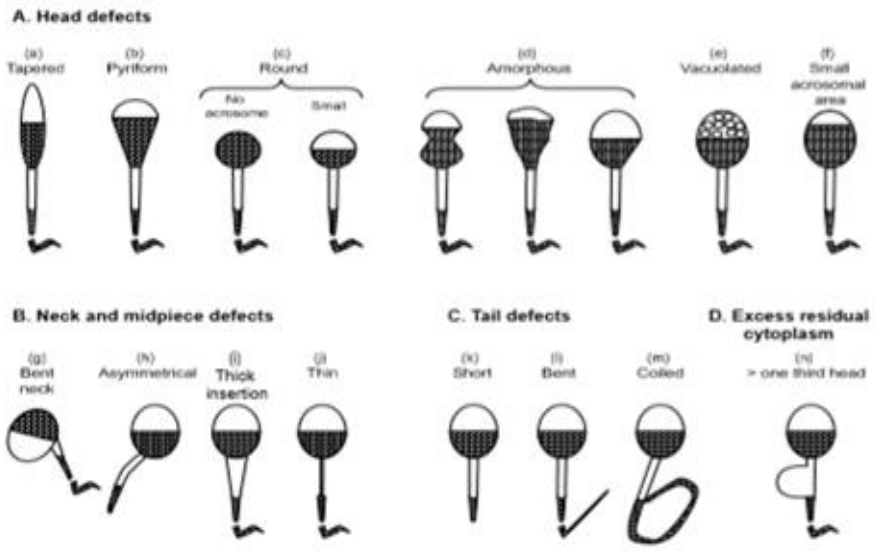

Figure 3. Abnormal spermatozoa morphology (5).

Head

Large or small, tapering, bulb-shaped / pyriform, amorphous, hollow >20% of the head area is occupied by a cavity that is not stained, the head is broad and a combination of the above.

Midpiece

Midpiece defects includes tailless spermatozoa that appear as free heads, or loose heads, uninserted tails or swollen/irregular central bent tails. Abnormally thin midsection e.g. no

mitochondrial sheath or various combinations of these abnormalities.

Tail

Short tails, double tails, shaped like hairpins, broken, curled tails with drips at the ends, or a combination of these abnormalities.

Automatic Method

The tool used is the Lenshooke™ SQA X1 PRO, a product from Bonraybio, a device that works automatically for human semen analysis by integrating mechanical, optical, electronic and algorithmic technologies. With the use of this Semen Quality Analyzer, it will facilitate and improve performance in the laboratory so that work is more efficient. Semen analysis using equipment equipped with a computer is an automated method that can provide objective and precise information about the characteristics of semen samples, such as morphology, concentration, and motility (16). Quality Control of the Cement Quality Analyzer can be standardized for each tool, so as to minimize differences in the results of cement analysis between laboratories. Semen analysis in combination with computer technology has evolved over the past 40 years, through advances in devices for capturing images from microscopes, massive increases in computing power along with tremendous reductions in computer size, new computer languages, and updated software algorithms (17).

In this study, the automatic tool used is the Lenshooke™ SQA X1 PRO, a product from Bonraybio, a device that works automatically for human semen analysis by integrating mechanical, optical, electronic and algorithmic technologies.

The working principle of the Lenshooke™ SQA X1 PRO tool is with a beam of light on the object of the examination, and then a high-resolution camera, with an optical lens facility, will take pictures of the object. Furthermore, the clinical database that has been recorded by the camera is analyzed for calculations with the algorithm (15).

The initial rare Work Procedure prepares the sample by putting this sperm collection device for the Semen Quality Analyzer using a special consumable cup test, and then wait 30 to 60 minutes for sperm to thaw. Homogenized the sample in the cup by turning it back and forth 8 – 10 times, check the color and volume of the sperm sample (Figure 4-5).

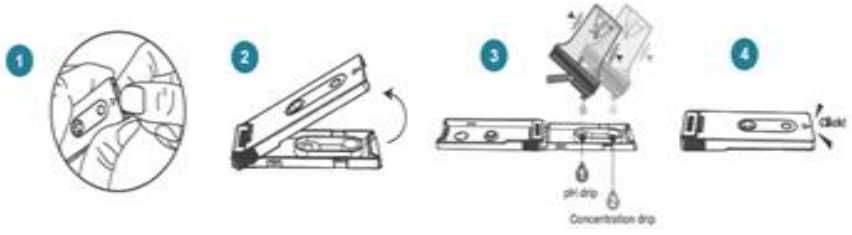

Figure 4. How to operate the Lenshooke™ SQA X1 PRO appliance (11).

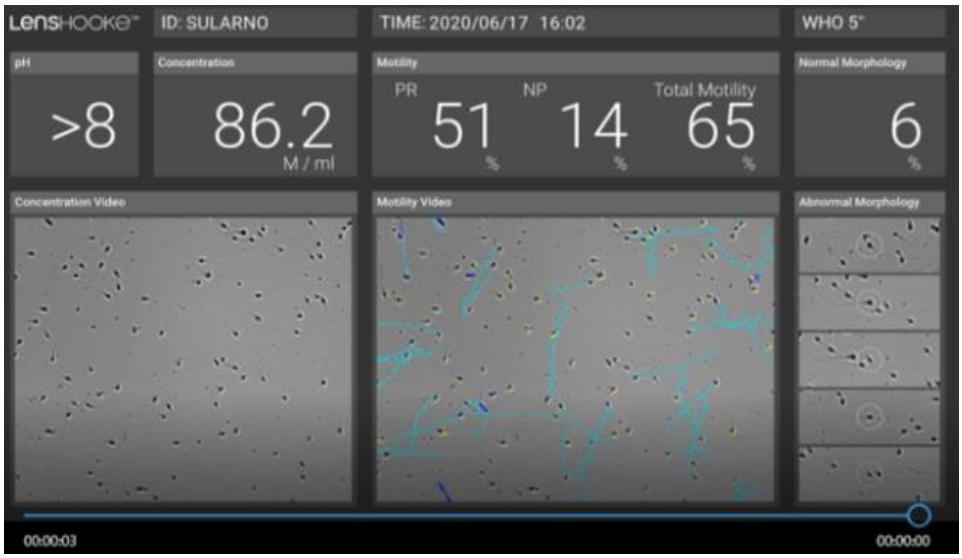

Figure 5. Display results on the Lenshooke™ SQA X1 PRO tool showed pH of the semen, total spermatozoa in million per mililiters semen, total moved of the spermatozoa, and amount of the total normal morphology of the spermatozoa (11).

Spermatozoa morphology parameters that emerged from readings using the Lenshoke™ SQA X1 PRO device were normal morphology with units of percent. Abnormalities in sperm size and morphology assessment includes the percentage of head length, head width, head circumference, head area and tail length (15).

RESULTS

Conformity Test

The suitability of the results of the automatic method of spermatozoa morphology examination with manual method of spermatozoa morphology using the 2 x 2 Contingency table and the Chi Square test. The characteristic and examination result are shown in Table 1 and 2.

Referring to WHO 2010 standard value of normal spermatozoa morphology 4%. The conformity test used a 2 x 2 contingency table from normal morphological data (Table 3). The results of this study could not be analyzed. Because of the 36 samples tested by the manual method, all showed normal results. This results in abnormal morphological data of 0. The agreement that can be made with the 2010 WHO standard normal value ≥ 4% is by looking at the percentage of automatic normal results compared to manual reaching 94.4% and high and low yields from both methods illustrate that the average the automatic method shows that the normal results are lower than the manual method shown as 83.3%.

Clinical Based Suitability

According to the 2010 revision of WHO guidelines, men with normal sperm cell

morphology is ≥4%. This is observed by namely by macroscopic and microscopic assessment of the semen (Table 4-5). The sperm concentrationof normal results automatically compared to manual, reaching 94.4% and the high and low results of the two methods illustrating that the average automatic method results show more normal results lower than the method. manual, namely 83.3%, as shown in Table 6.

Table 1. Subject Characteristics

Age (Year)	Σ	Sampling Technique	Σ	Abstinence (Day)	Σ
21 - 30	23	Masturbation	48	<2	0
31- 40	20	Coitus Interruptus	0	2 – 7	46
> 40	5	Special Condoms	0	>7	2
amount	48		48		

Table 2. Exclusion Research Samples

Description	Σ
Abstinence for more than 7 days	2
The sample is red / mixed with blood	1
Liquifaction more than 60 minutes	2
The concentration of spermatozoa is less than 2 million	6
Reagent Cassette damaged reading part is subject to hand grease	1
Amount	12

Statistical Based Conformity

Conformity Standard Mean between Automatic Normal Morphology and Manual Normal Morphology

Table 3. Contingency 2x2 mean normal spermatozoa morphology

Automatic Normal Mean * Manual Normal Mean					
			Mean Normal Manual		**Total**
			Positive	**Negative**	
Automatic Normal Mean	**Positive**	Count	10	1	11
		% of Total	27.8%	2.8%	30.6%
	Negative	Count	7	18	25
		% of Total	19.4%	50.0%	69.4%
Total		Count	17	19	36
		% of Total	47.2%	52.8%	100.0%

Testing the results of research based on statistics using the standard mean value of the data;

- Normal Morphology automatic method and Normal Morphology manual method.
- Abnormal Head area automatic method and Abnormal Head area manual method
- Abnormal Tail Length automatic method with Abnormal Tail length manual method.

a. Sensitivity = 10/17 = 0.588%
b. Specificity = 18/19 = 0.947%
c. Positive prediction value = 10/11 = 0.909%
d. Negative prediction value = 18/25 = 0.720%

Table 4. Suitability test for the mean morphology of normal spermatozoa

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	12,130 [a]	1	0.000		
Continuity Correction	9,737	1	0.002		
Likelihood Ratio	13,446	1	0.000		
Fisher's Exact Test				0.001	0.001
Linear-by-Linear Association	11,793	1	0.001		
N of Valid Cases	36				

The suitability test is based on the calculation of the mean morphological value of spermatozoa with significance 0.001 <0.05. It can be concluded that H_0 is rejected and H_1 is accepted, which means that by means of statistical analysis, there is a difference between automatic spermatozoa morphology and manual spermatozoa morphology (Table 7).

Standard Conformance Mean Between Automatic Abnormal Head Area and Manual Abnormal Head

Spermatozoa with slightly abnormal 'borderline' heads are classified as abnormal. The suitability test was based on the calculation of the mean morphology of abnormal spermatozoa head with a significance of 0.956 > 0.05 (P < 0.05; r = -0.10). Then by means of statistical analysis, we can observe that H_0 is accepted and H_1 is rejected, which means there is no difference between the calculation of abnormal morphology of spermatozoa head automatic method with abnormal sperm morphology head manual methods.

Table 5. Contingency 2 x 2 mean abnormal head morphology

Mean Head Area * Mean Head Manual					
			Mean Head Manual		**Total**
			Positive	**Negative**	
Mean Head Area	**Positive**	Count	7	10	17
		% of Total	19.4%	27.8%	47.2%
	Negative	Count	8	11	19
		% of Total	22.2%	30.6%	52.8%
Total		Count	15	21	
		% of Total	41.7%	58.3%	

a. Sensitivity = 7/15 = 0.467%
b. Specificity = 11/21 = 0.524%
c. Positive Predicted Value = 7/17 = 0.412%
d. Prediction Value Negative = 11 / 19 = 0,579%

Table 6. Suitability test for mean morphology of normal spermatozoa

	Value	df	Asymp. Sig. (2-sided)	Exact Sig (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.003	1	0.955		
Continuity Correction	0.000	1	1,000		
Likelihood Ratio	0.003	1	0.955		
Fisher's Exact Test				1,000	0.611
Linear-by-Linear Association	0.003	1	0.956		
N of Valid Cases	36				

The suitability test was based on the calculation of the mean morphological value of abnormal spermatozoa tail with a significance value of 0.339 > 0.05. Hence, by means of statistical analysis, it can be concluded that H0 is accepted and H1 is rejected, which means that there is no difference between the calculations of the abnormal spermatozoa tail morphology of the automatic method.

Standard Conformance Mean between Automatic Abnormal Tail and Manual Abnormal Tail

Table 7. Contingency 2 x 2 mean abnormal morphology of tail

Automatic Tail Mean * Manual Tail Mean					
			Manual Mean Equipment		**Total**
			Positive	**Negative**	
Automatic Tail Means	**Positive**	Count	8	9	17
		% of Total	22.2%	25.0%	47.2%
	Negative	Count	12	7	19
		% of Total	33.3%	19.4%	52.8%
Total		Count	20	16	36
		% of Total	55.6%	44.4%	100.0%

a. Sensitivity = 8/20 = 0.400%
b. Specificity = 7/16 = 0.438%
c. Positive Prediction Value = 0.471%
d. Negative prediction value = 7/19 = 0.368%

Table 8. Contingency 2 x 2 mean abnormal morphology of tail

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.942	1	0.332		
Continuity Correction	0.403	1	0.526		
Likelihood Ratio	0.945	1	0.331		
Fisher's Exact Test				0.503	0.263
Linear-by-Linear Association	0.916	1	0.339		
N of Valid Cases	36				

DISCUSSION

This research was conducted at the Clinical Pathology Laboratory of Mother and Child Hospital "Restu Ibu" Sragen from June 2020 to August 2020. Researchers carry out the rules of practice in the laboratory examination stages as should the Pre-Analytic, Analytic and Post-Analytic.

Overall in this study, researchers conducted 48 patient samples, of which 12 patient samples were included in the exclusion criteria. 36 patient samples entered the inclusion criteria according to the needs of the sample in this study. The sample characteristics of the 36 samples studied showed as many as 20.

At the stage of carrying out the analysis using the Leenshoke SQA X1PRO automatic tool, there are several things that need to be considered to get the results according to the patient's clinical condition, namely: measure the use of electricity used for automatic devices is stable, perfect sample homogeneity, the cleanliness of the cassette in the reading lens area must be completely clean, free of grease and dirt, cross checking reads using the manual method on samples with low spermatozoa concentrations. Because at concentrations below 2 million spermatozoa readings on the automatic instrument will be read with a result <1 for concentration, morphology and motility, and then immediately remove the cassette from the instrument after the reading is complete.

In the suitability test of normal morphological results with automatic methods compared to manual methods as Gold standard, using 2x2 contingency tables to see sensitivity, specificity, normal predictive values and abnormal predictive values. With the normal standards set by WHO 2010, cannot be tested with 2x2 contingency tables. Because the abnormal value in the manual method is 0. All results from the 36 samples from the manual method fall within the normal value range of ≥4 million/mL.

Automatic morphological comparisons that can be done clinically with WHO 2010 standards by looking at the number of normal results achieved by the automatic method of the 36 samples studied. The achievement was 34 samples entered the normal range. This means that this automatic achievement

compared to the manual method reaches 94.4%. The normal morphological average yield of the automatic method is lower than the manual method. Data shows that of the 36 samples, 83,3% lower than the manual method.

Because the standard normal value set by WHO 2010 cannot be clinically tested using a 2 x 2 contingency table, the automatic method suitability test is performed statistically by determining the standard of the mean value. The statistical test consisted of data on normal morphology, abnormal morphology of the head and abnormal morphology of the tail. After testing the three data with the standard mean as standard, the following results are obtained:

a. Normal morphological data shows a significance value of 0.001 < 0.005, it can be concluded that H_0 is rejected and H_1 is accepted, which means that there is a difference between statistical calculations for spermatozoa morphology with automatic method and manual method of spermatozoa morphology.
b. Data on abnormal morphology of the head had a significance value of 0.371 > 0.05. It can be concluded that H_0 is accepted and H_1 is rejected, which means that statistically there is no difference between the calculation of abnormal morphology of spermatozoa head automatic method with abnormal sperm morphology manual methods.
c. Data on abnormal morphology of tails with a significance value of 0.339> 0.05. It can be concluded that H_0 is accepted and H_1 is rejected, which means that statistically there is no difference between the calculation of abnormal spermatozoa tail morphology automated method with abnormal sperm morphology manual methods.

The presence of an automatic tool Lenshooke™ SQA XI PRO, has its advantages such as simple physical tools, easy operation with touch screen technology, quick reading of results that only takes 3 to 5 minutes, visualization of spermatozoa in the form of a video, focus setting and HDMI connection to the monitor can display pH, motility, concentration and morphology. Disadvantages of this device are in conditions of low spermatozoa concentration, the device cannot read spermatozoa completely, and the investment costs for equipment and operational costs for reagents are quite high compared to manual ones. In the manual method with Papanicolaou dye, the results of the head, tail and cytoplasmic morphology of spermatozoa were more clearly read than other dyes. Tool investment costs and operating costs are lower than automated methods. The data from the research were carried out by descriptive tests with the results of the standard deviation of the morphology of normal or abnormal spermatozoa, the manual method showed the

results with the same value, namely 2.253 and the standard deviation value of the morphology of normal / abnormal spermatozoa, the automatic method also showed the same results, namely 2.247.

In the suitability test for normal morphology results, the automatic method is compared to the manual method as the Gold standard, using a 2x2 contingency table to see the sensitivity, specificity, normal predictive value and abnormal predictive value, with the normal standard set by WHO 2010, cannot be tested with a 2x2 contingency table because the abnormal value in the manual method was 0. All the results from 36 samples of the manual method were within the normal value range of 4 million/mL. The automatic morphological comparison that can be done clinically with the 2010 WHO standard is by looking at the number of normal results achieved by the automated method. Of the 36 samples studied, the achievement is that as many as 34 samples are in the normal range. This means that if the automatic achievement is compared to the manual method, it reaches 94.4%. The average yield of the normal morphology of the automatic method is lower than that of the manual method. The data shows that of the 36 samples, 83.3% lower than the manual method.

CONCLUSION

Spermatozoa morphology examination is important in terms of determining a man's overall fertility potential. Morphology examination take into observation multiple aspects, namely a sperm's shape such as its head, midpiece, tail, and the presence of cytoplasmic droplets. The most common Spermatozoa morphology examination in medical laboratory is microscopic observation by manual and eutomatic machine. In general, the presence of the Lenshooke^{TM} SQA XIPRO device which is based on automatic technology is compatible with the manual method. Hence, this automated tool can be used in laboratory services complementing the manual method as the Gold Standard.

AUTHOR CONTRIBUTIONS

All author has equal contribution for the study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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