A Rapid and Reproducible Report on the Morphological and Functional Characteristics of Spermatozoa Using Digital Image Processing Technique

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Abstract

Background and Objectives: Semen analysis plays a vital role in understanding the healthy state of the sperm in men. The computer aided semen quantification technique quantifies the quality of the sperm from the semen sample which is digitally sampled and processed using digital image processing technique.
Methods: The semen samples were collected from 402 infertile men aged between 25-50 years. Similarly 25 samples were collected from the age matched healthy fertile men (control group) as per the diagnostic report from the physician. A total of 427 samples used in this study were analyzed using traditional manual method (ground truth) and the proposed automated method based on the image processing algorithm.
Results: Conventional semen analysis procedure was performed manually after liquefaction of the samples. The parameters such as morphology, sperm count and motility types were determined and compared between manual and automated methods. We have achieved a significant repeatability and reproducibility of the results using the automated method. Automated method has demonstrated to be computationally efficient and it required less amount of time to process any given field of view. It is also less susceptible to any rater bias for the analyzed field of view and the results were comparable with the manual method.
Conclusions: In this article we describe the developmental stages involved in the semen analysis, custom built automated image analysis protocol and the report generation based on the parameters involving sperm count and motility types.

Keywords

Semen analysis, sperm count, sperm motility, image processing

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1 Introduction

The responsibility of infertility falls on both male and female factors, a single and major male factor involved in infertility associated with spermatozoa [1]. Evaluation of sperm count, morphology and its motility are very useful factors in the diagnosis of male infertility. Manual method of semen analysis is rather subjective technique, time consuming and often show large inter-laboratory variations [2]. Computer based sperm analysis methods are classical ways to determine the potential fertility of boars [3]. The four major factors which have to be considered during the evaluation of boar are sperm quality, concentration, motility and acrosome integrity [4]. Image processing techniques were used for a rapid and reproducible report on the structural and functional evaluation of spermatozoa by fully automated methods. Computer-Assisted sperm analysis systems provide a rapid and automated assessment of the sperm characteristics, together with improved standardization and quality control [5].

In this work, the spermatozoa and its motility were captured using high resolution digital video camera. The captured video was converted in to image frames and was subjected to object tracking image processing algorithms to study the sperm structural and functional characteristics [1]. Sperm motility was studied according to

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the guidelines from World Health Organization (WHO) [6]. The specific morphological characteristics such as, the count and the percentage of motility of the individual spermatozoa were assessed. The procedure was repeated with different samples and the results were recorded. Aliquots of the same semen samples were analyzed simultaneously by manual method. The results were recorded for both manual and the computer assisted method for further validation.

2 Materials and Methods

The samples were collected freshly after 3 days of absitinance period by strictly following standards of the World Health Organization. These samples were collected from infertility clinic (Rajah Muthiah Medical College Hospital) between the periods from February 2008 till March 2010. A total 427 samples were collected, among them 402 were infertile and 25 are healthy controls with the mean age of 37.5 ± 12.5.

2.1 Conventional Semen Analysis

It was clearly explained to the individuals concern, about cleaning the hands, external genitalia and the collection procedure of semen. The samples were collected by masturbation after passing the urine. The ejaculated semen was collected in a sterile wide mouth container and the time of collection was recorded. Manual semen analysis was carried out with diluted semen in saline in the proportion of 1:20 (0.02 mL specimen mixed with 0.38 mL of saline), after liquefaction at a temperature of 37°C for 30 minutes. The morphology, motility, and parameters such as the percentage of actively motile, sluggishly motile and non-motile were observed using a wet mount preparation using high power objective. The total sperm count was recorded using Neu baur Chamber after appropriate dilution with semen diluting fluid.

2.2 Computerised Semen Analysis

The same samples were analyzed using computer based semen analysis tool developed in-house for this project. A wet mount was made using a drop of semen and focused with high power objective of a trinocular microscope. The video image of the sperms was captured using high resolution (8MP) digital camera with a frame rate of 25fps PAL format fitted on the trinocular head of the microscope. Using the video mode, the motility of the spermatozoa was recorded. The illumination and brightness of the field of view were kept uniform, to minimize the segmentation error. The recorded video was processed sequentially and each frame is considered as a single digital image. The digital images were fed to the object tracking algorithm (based on image indexing method) developed in MATLAB environment. For each frame the segmentation of the sperms were done as the initial step. Debris in the frame was removed easily since the signal intensity of the debris was not in the range of sperm. The Matlab based image indexing method was used to separate different objects (sperm, debris and background) in the image according to their intensity distribution. The indexing method clusters the image intensities according to the number of input clusters given to the algorithm. These indexed sperms were segmented and tracked on the neighboring frames around a particular block of neighborhood. The distance travelled by a particular sperm over the number of frames enable to determine their types of motility. The Euclidean distance was used to measure the distance travelled by the sperm between the first and the last frame and their velocity (distance*time (number of frames in this case)) had been estimated to classify the sperm types. The parameters such as number of sperms in a frame, motility (active motile, sluggish, non-motile), total count and their percentage were calculated for each field of view. The ratio of number of sperms to the distance travelled aided to classify the indexed sperm into actively motile, sluggishly motile and non-motile sperm. The intermediate group between the motile and non-motile is referred as sluggishly motile.

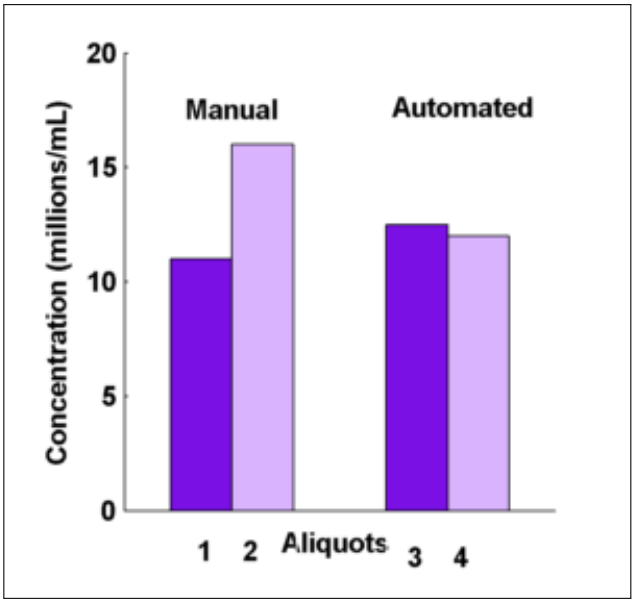

Figure 1: Comparison of total sperm count in Oligospermic semenbetween manual and automated methods.

3 Results

High resolution image of the spermatozoa in the selected field of view helps to reduce the background and to extract the clear boundary of the sperm. The sperms present on each frame were segmented from the given field of view initially and each of the frames was processed sequentially, which enabled to track the motion of individual sperms from frame to frame. By calculating the distance travelled by each sperm over a large dataset of frames for a definite period of time we were able to determine the

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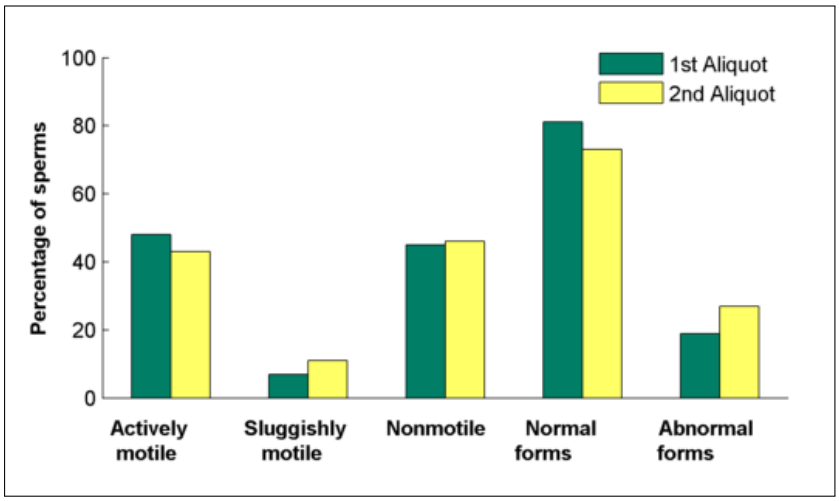

Figure 2: Percentage of sperms classified with respect to their type of motility using manual method for Oligospermic semen analysis.

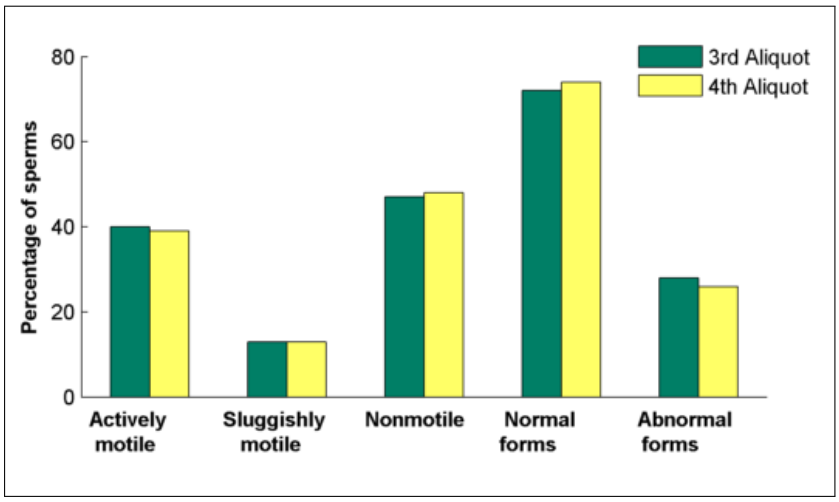

Figure 3: Percentage of sperms classified with respect to their type of motility using proposed automated method for Oligospermic semen analysis.

motility associated with each sperm. Based on the derived parameters an exact quantification of sperm count, and their motility characteristics were determine. The ratio of motile sperms to the total number of sperms in the given field of view for the dataset enables to classify the dataset into Azospermic, Oligospermic and Normospermic and compared with the manual methods.

The results of Oligospermic semen analysis using the automated method is comparable to the results obtained by manual method. We have observed larger difference in the total sperm count in 1st and 2nd aliquot using the manual method. In automated method we observe that the results were reproducible, the difference in the 3rd and the 4th aliquot is significantly lesser than the difference obtained in manual method. The difference observed in the automated method might be contributed by artifacts occurred during the video acquisition. However with second aliquot the values of various parameters observed in the manual method were not consistent with the 1st aliquot (Figure 2), whereas the automated analysis yielded almost a similar result both in the 3rd and 4th aliquot (Figure 3). The variations in the total sperm count in Oligospermic semen analysis are illustrated in Figure 1 for manual and automated methods. Similarly Figure 2 and 3 explain the intra assay differences of additional parameters such as motility and abnormal forms in the manual and automated methods respectively.

The difference exhibited with respect to the number of sperms is clearly illustrated in Figure 4 for both manual and automated methods. The results of Normospermic semen analysis proved that the automated method of sperm count is comparable with the manual method (Table: 1, aliquots 1 and 3). While comparing the 2 aliquots of manual method we could observe the variability of the parameter between the aliquots. This variation between the aliquots (Table: 1 3rd and 4th aliquot) is not highly pronounced in the automated method.

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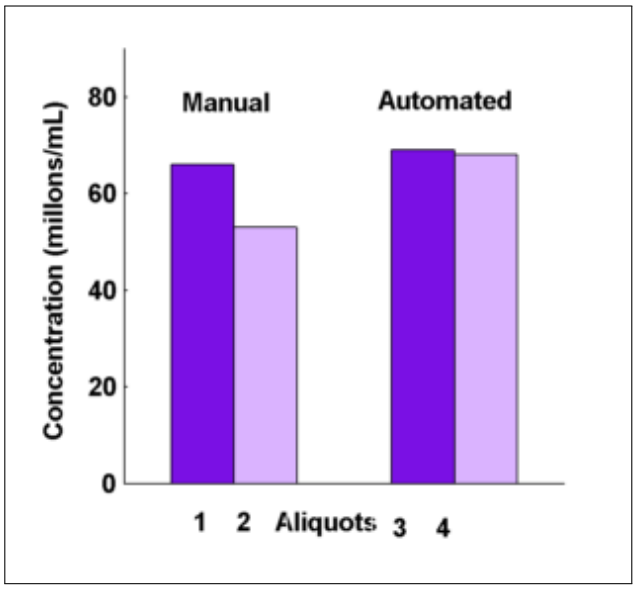

Figure 4: Comparison of total sperm count in Normospermic semen between manual and automated methods.

Similarly Figure 5 and 6 explain the intra assay differences of additional parameters such as motility and abnormal forms in the manual and computerized methods respectively. It was observed both in primary and in repeated analysis of the same Normospermic semen by computerized method showed almost consistent results, whereas in the manual method have large variations and are highly subjective.

In-house custom build software along with graphical user interface (GUI) was designed specifically for this application in Matlab shown in Figure 7. The GUI interface contains the provision for loading the Image and running the object tracking algorithm to calculate the motility of the sperm and displaying the end results which can be useful in day today routine clinical practice.

4 Discussion

The 427 study sample was divided into four equal aliquots. After incubation for liquefaction, aliquots of the same sample were simultaneously subjected to both conventional and automated semen analysis procedure. Aliquots one and three were analyzed by conventional and automated method respectively. The same procedure was repeated again for second and fourth aliquots to test for reproducibility.

It was reported by Fereidoon et al., that the results by human are prone to minimal errors and proved to be a time consuming in a conventional sperm analysis performed by a specialist under light microscope [1]. Slow moving cells other than the motile sperms, image of certain debris and immotile sperms from which the sperms must be differentiated which is not possible in manual method causing these errors [7]. It is also difficult for the specialist to keep track of all the moving objects and the stationary debris at the same instant and it leads to significant stress in the eyes of an observer. Therefore manual method is rather subjective technique, time consuming and often contributes to large inter- laboratory variations [6] leading to difficulties in achieving the desired reliability.

High quality input images are vital for the image processing algorithm developed in MATLAB 6.5.1 software in windows XP operating system on a Pentium IV personal computer. With the above conditions being ideal, one could estimate and quantify the sperm count, the structural variations, and type of motility using automated methods. In this work the image of spermatozoa and its movement along with other parameters were analyzed using high resolution video camera fitted with microscope. Parameters such as sperm motility, type of motility, normal and abnormal forms of the above two methods were compared. Results of semen analysis by manual method were consistently difficult to maintain the reproducibility.

Normal and abnormal classifications were made based on the results of the semen samples and the samples were classified as Azospermia, Oligospermia and Normospermia. On analysis of the repeated results on the parameters of above three classes, we could able to observe a consistent results both in Oligospermic and in Normospermic semen using automated method. The values of the parameters estimated by manual method were inconsistent. Preliminary study results illustrates that the use of automated semen analysis is more objective and reproducible than technician based motility assessment. In future we are aiming to capture the structural pattern and morpho-

Table 1: Normospermic semen analysis between manual and automated method.

Parameters analyzed	Manual method		Computerized method	
	1st aliquot	2nd aliquot	3rd aliquot	4th aliquot
Total sperm count	66 millions/ml	53 millions/ml	69 millions/ml	68 millions/ml
Actively motile (%)	70	61	78	77
Sluggishly motile (%)	12	19	10	12
Non motile (%)	18	20	12	14
Normal forms (%)	83	81	78	80
Abnormal forms (%)	17	19	22	20

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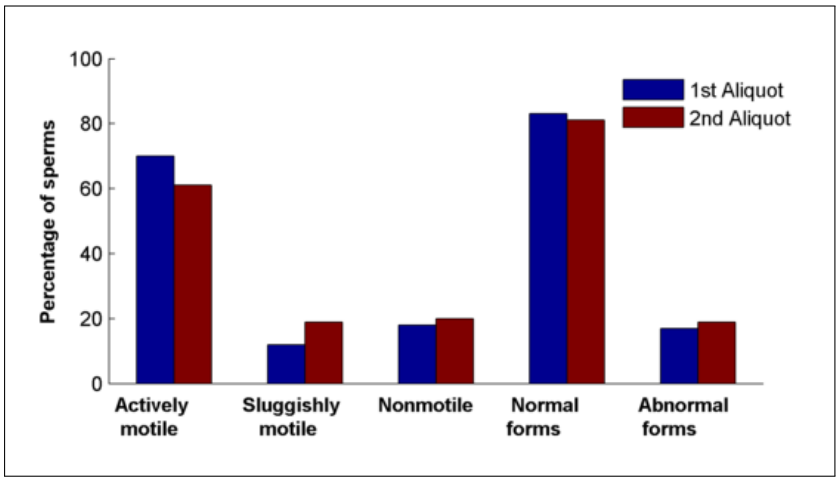

Figure 5: Percentage of sperms classified with respect to their motility using manual method for Normospermic semen analysis.

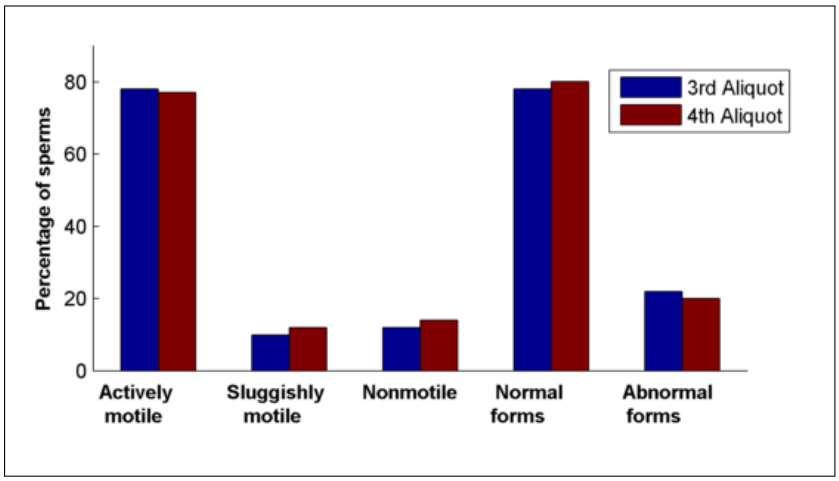

Figure 6: Percentage of sperms classified with respect to their motility using automated method for Normospermic semen analysis.

logy of the sperm and estimate a feature space to segment all types of sperms which can provides a better insight of the structural and functional characteristics of the sperm.

5 Conclusion

In conclusion automated semen analysis provides objective results in minutes along with the report and its user interface allow performing a complex operation in a simpler way.

The physicians reviewed about its convenience to handle multiple datasets in a given instant and its easier way of generating the reports. On repetition of the experiment on different samples the results were obtained with acceptable variations.

Results were encouraging in Oligospermic and Normospermic samples. When the count was more than 80 million/ml (study under process results not given) a dilution technique was needed to reduce the overcrowding of spermatozoa to get reproducible results. We propose to further develop and enhance the features of this user-friendly sperm analysis method with increasing the number of parameters like morphology, velocity of the sperm in different concentrations, which will give a better understanding of the underlying principles of motility.

References

[1] Fereidoon N.R., Mohammed H.M and Vahid R.N. A Multi Steps Algorithm For Sperm Segmentation In Microscopic Image. Proceedings of the World Academy of Science, Engineering and Technology. 2005; 12: 43-45.

[2] Jorgensen N., Auger J., Giwercman A., Irvine D.S., Jensen T.K., Jouannet P., Keiding N., Le Bon C., MacDonald E., Pekuri A.M., Scheike T., Simonsen M., Suominen J., and Skakkeboek N.E. Semen Analysis Performed By Different Laboratory Teams: An Intervariation Study. Int.J.Anrol. 1997; 20(4): 201-208.

[3] Sanchez L., Petkov N., and Alegre E. Statistical Approach To Boar Semen Evaluation Using Intracellular Intensity Distribution Of Head Images. Cellular and Molecular biology. 2007; 52(6): 38-43.

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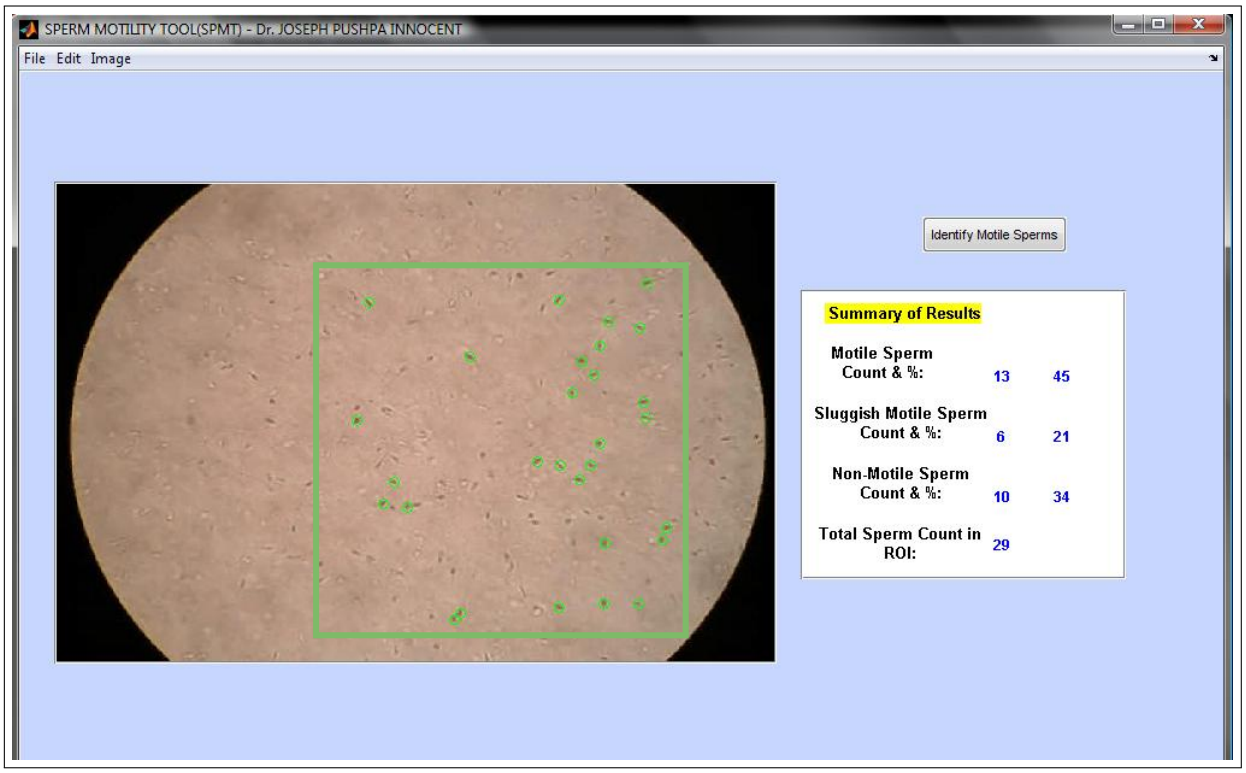

Figure 7: Graphical user interface for Sperm motility analysis. (Region of Interest to be analysed is manually marked by rectangle box in light green by the user on one frame to perform the automated segmentation in all the remaining frames; the identified sperms are marked in dark green).

[4] Thurston L.M., Holt W.V., and Watson P.F. POST-Thaw Function Status Of Boar Spermatozoa Cryopreserved Using Three Controlled Rate Freezers: A Comparison. Theriogenology. 2003; 60(1): 101-113.

[5] Vahid R.N., Mohammad H.M., and Mohammad H.N. Sperm Identification Using Elliptic Model and Tail Detection. Proceedings of the World Academy of Science, Engineering and Technology. 2005; 6:205-208.

[6] Lene L., Thomas S., Tina K.J., Jens P.B., Erik E., Niels H.H., Yue Z., and Niels E.S. Computer–Assisted Semen Analysis Parameters As Predictors For Fertility Of Men From The General Population. Human Reproduction. 2000; 15(7): 1562- 1567.

[7] Jens P.B., Erik E., Tina K.J., Niels H.H., Henrik K., Thomas S., Aleksander G. and Niels E.S. Relation Between Semen Quality And Fertility: A Population Based Follow-Up Study Of 430 First Pregnancy Planners. The Lancet. 1998; 352: 1172-1177.

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