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ORIGINAL ARTICLE

Performance Evaluation of KU-F40 Automatic Feces Analyzer

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SUMMARY

Background: **The performance of a new inspection system should be verified before it is used. The aim of this study was to analyze the performance of the KU-F40 automatic feces analyzer, which can serve clinic better.**
Methods: **A total of 1,822 fecal samples were collected at our hospital in September 2022 and tested by manual method and KU-F40 automatic feces analyzer. With manual microscopy as the gold standard, the clinical application value of KU-F40 was evaluated by sensitivity, specificity, coincidence rate, etc.**
Results: **For the KU-F40 automatic feces analyzer, the sensitivity of red blood cells (RBC), white blood cells (WBC), fungi, and occult blood (OB) was 95.08%, 92.68%, 89.47%, and 98.72%, respectively. The specificity was 99.38%, 99.83%, 99.94%, and 99.04%, respectively. The coincidence rates were 99.23%, 99.51%, 99.84%, and 98.96%, respectively. The Kappa values were 0.8883, 0.9415, 0.9181, and 0.9729, respectively. The p-values were 0.0574, 0.5078, 1.0000, and 0.1671, respectively.**
Conclusions: **KU-F40 automatic feces analyzer results are reliable, can effectively improve the efficiency of testing, reduce the risk of biosafety, which is accordance with the current clinical need. At the same time, continuous improvement should be made to better serve the clinic.**
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KEYWORDS

feces, fecal examination, automatic feces analyzer, KU - F40, evaluation of performance

INTRODUCTION

Feces is the product of food digested and absorbed by the body, which is of great value for the screening of gastrointestinal inflammation, bleeding, bacterial and parasitic infections, and tumors [1-3]. Up to now, the main methods of fecal examination are physical, chemical, and microscopic examination. The traditional method used sensory examination to examine the physiological properties of feces, that is, color and character. Occult blood is examined by chemical or immunochemical method, and morphology is examined by direct smear microscopy, but the traditional method is difficult for the examiners because of the smell and character of the specimen. More importantly, with the global epidemic of the novel coronavirus in recent years, there are more reports that the novel coronavirus (SARS-CoV-2) has

been found in feces [4,5], leading to the enhancement of people's awareness of biosafety and that of higher requirements for the laboratory. The automatic feces analyzer has gradually attracted the attention of laboratories. At the same time, with the development of science and technology, the feces analyzer combined with the development of artificial intelligence has been constantly updated and its performance has been gradually improved. The automatic fecal analyzer made by Zhuhai KEYU Biological Engineering Co., LTD (hereinafter referred to as "Zhuhai KU") has been developed and updated from KU-F10 to the 4th generation KU-F40. This machine is a new-generation stool analyzer created by KEYU Company based on the counting of optical flow counting pool, automatic microscope control, and AI recognition technology, auto-focus without focusing liquid, layered stereoscopic photography and CMOS image processing, etc. [6]. Now, the performance of KU-F40 is analyzed comprehensively and its clinical application compliance is evaluated [7].

MATERIALS AND METHODS

Clinical data

In September 2022, 1,822 stool samples were collected from outpatients, inpatients, and physical examination patients in our hospital, including 176 outpatients, 1,523 inpatients and 123 physical examination patients. There were 1,080 males and 742 females. The age range was from 1 day to 97 years with a mean age of 45.5 years.

Instruments and reagents

KU-F40 automatic feces analyzer, corollary reagents, and quality control products. OB test reagent was occulted blood colloidal gold test strip produced by Beijing Wanhuapman Biological Engineering Co., LTD. (Regent Lot: 22020027). Nikon eclipse E200 optical microscope, Japan.

Methods

Before the performance evaluation of the KU-F40 analyzer starts, the relevant parameters of the analyzer need to be set and fixed. In other words, all the results of the equipment evaluation are generated based on the relevant settings of these parameters. Due to the numerous parameters of the instrument settings, only a few main parameters are listed here, as shown below Table 1. At the same time, the evaluation details are made according to the industry standard of the People's Republic of China YY/T 1745-2021 automatic feces analyzer.

Detection limit of RBC and WBC

Prepare red and white blood cells with a concentration of about 10/μL according to the method given in Appendix A of YY/ T1745-2021, and measure them 20 times by the normal test model on the instrument. When the number of statistical results is greater than 0 it is counted as N, the detection rate is N/20*100%, and the detection rate shall be ≥ 90%.

Carry pollution rate

Prepare high concentration cell samples (within the analytical measurement range) and normal saline according to the method given in Appendix A of YY/ T1745-2021. First, the cell samples with high concentration were tested three consecutive times, and the test results were i1, i2, and i3. Normal saline was tested 3 times, and the test results were j1, j2, and j3. The calculated Carry pollution rate = (j1-j3)/(i3-j3) * 100%. The calculated Carry pollution rate of the analyzer must be less than 0.05%.

Precision of the morphology components

Within-run precision: cell specimens with concentrations about 50/μL and 250/μL were prepared and tested 20 times each. The coefficient of variation (CV%) was calculated according to the obtained data. Between-day precision: 20 consecutive days of indoor quality control data was used to evaluate the between-day precision. When the cell concentration is 50 - 200/μL, the CV value must be less than 20%. When the cell concentration is more than 200/μL, the CV value must be less than 15%.

Accuracy assessment

This assessment is completed in terms of the comparison of instruments and manual: stool samples are tested by KU-F40 and manual microscopy, respectively. Meanwhile, manual microscopy is regarded as the gold standard, and manual microscopy personnel are required to be inspectors with medium-grade professional title or above. Manual microscopy was performed according to the National Guide to Clinical Laboratory Procedures, fourth edition. The KU-F40 is operated according to the Standard Operating Procedure file, and the fecal specimens should be placed in the collector attached to the KU-F40. The detection should be completed within 2 hours. The test results refer to the "National Guide to Clinical Laboratory Procedures": 1. Physical characteristics: brown or yellow, soft stool; 2. Red blood cells, fat globules, fungi, parasitic eggs and fecal occult blood, all these are negative; 3. White blood cells: absent or occasionally seen. It should be noted that for the assessment of parasitic eggs, due to the limitations of the samples, it is difficult to achieve the compliance assessment of large samples in a short time. Therefore, 6 cases of existing parasites were tested on the KU-40 to evaluate the detection and recognition of the analyzer.

Statistical methods

GraphPad Prism V8 statistical software was used for data analysis. Kappa test was used for the consistency of detection results by KU-F40 instrument method and manual method, and Kappa > 0.8 indicated that the re-

Table 1. Basic parameters of the KU-F40.

Project	Parameter setting
Maintenance - reagent - mixing time	8 seconds
Maintenance - focus - visible component settlement time	60 seconds
Advanced options - Photo parameters	group A: 16 photos group B: 32 photos
System Settings - Mixing motor	mixing speed: 70 RPM/s
Item test - morphology component item - red blood cells	confidence, high threshold, low threshold and count threshold were 0.9, 0.9, 0.8, 1
Item test - morphology component item - white blood cells	confidence, high threshold, low threshold, and count threshold were 0.94, 0.94, 0.8, 1
Item test - morphology component item - fungus	confidence, high threshold, low threshold, and count threshold were 1, 0.9, 0.9, 0

Table 2. Verification table of detection limits of RBC and WBC.

Sample No.	RBC results	WBC results
1	13	10
2	6	10
3	8	13
4	6	13
5	11	10
6	8	10
7	8	4
8	11	4
9	13	7
10	13	7
11	8	7
12	6	0
13	6	10
14	3	7
15	6	10
16	11	7
17	11	7
18	11	7
19	6	4
20	8	4
Rate of detection (%)	100	95

sults were highly consistent; 0.6 < Kappa ≤ 0.8 indicates moderate consistency; Kappa ≤ 0.4 indicates poor consistency. To the comparison, x^2 test was used between groups, and $p < 0.05$ showed a statistically significant difference.

RESULTS

Detection Limit Verification: Red blood cells and white blood cells with concentrations of 10/μL were prepared, and the detection limits of both were verified by following the method described in the "Detection limit of RBC and WBC“. The results were shown in Table 2.

Table 3. Assessment table of carry pollution rate of RBC and WBC.

Parameter	RBC concentration (/μL)	WBC concentration (/μL)
High value i1	4,479	5,281
High value i2	4,262	4,868
High value i3	4,343	5,024
Low value j1	0	0
Low value j2	0	0
Low value j3	0	0
rate of carry pollution (%)	0	0

Table 4. Assessment table of RBC precision.

Sample No.	Within-run precision		Between-day precision	
	low value (50 - 200/μL)	high value (> 200/μL)	low value (50 - 200/μL)	high value (> 200/μL)
1	65	285	44	204
2	65	253	41	220
3	58	250	41	275
4	58	269	46	252
5	50	262	49	255
6	55	332	41	220
7	55	251	49	255
8	65	252	44	224
9	67	303	39	252
10	77	335	36	249
11	71	291	49	316
12	77	256	41	255
13	65	236	46	310
14	48	294	34	259
15	51	319	36	259
16	64	322	34	243
17	55	236	39	217
18	51	264	23	294
19	55	289	29	275
20	71	313	31	233
MEAN	61.15	280.60	39.6	253.35
SD	8.80	32.00	7.05	30.07
CV (%)	14.4	11.4	17.81	11.87

Carry pollution rate: Red blood cells and white blood cells were prepared with a concentration of about 5,000/μL in normal saline. The carry pollution rate of red and white cells was evaluated according to the method of 1.3.2. The evaluation data are shown in Table 3 below.

Precision assessment of morphology components: In accordance with Section 1.3.3 of the method, the intra-

Table 5. Linear evaluation of RBC and WBC.

Dilution ratio	RBC value	WBC value
1	6,041	7,278
1:1	3,070	3,357
1:2	1,263	1,524
1:4	769	730
1:8	364	418
1:16	179	208
1:32	138	150
1:64	83	58
1:128	45	26
1:256	26	15
The linear function	Y = 6,014.4x	Y = 7,098.2x
R value	0.9990	0.9980

Table 6. Comparison of instrument method and manual method.

Items	The number of samples	Both positives	Both negatives	MMN & INP	MMP & IMN
RBC	1,822	58	1,750	11	3
WBC	1,822	76	1,737	3	6
Fungus	1,822	17	1,802	1	2
Occult blood	1,822	463	1,340	13	6

MMN & INP - Manual method negative & instrument method positive, MMP & IMN - Manual method positive & instrument method negative.

Table 7. Performance evaluation of instrument method.

DI	Sensitivity	Specificity	PPV	NPV	FP	FN	CR	Kappa value	p-value
RBC	95.08	99.38	84.06	99.83	0.62	4.92	99.23	0.8883	0.0574
WBC	92368	99.83	96.20	99.66	0.17	7.32	99.51	0.9415	0.5078
Fungus	89.47	99.94	94.44	99.89	0.06	10.53	99.84	0.9181	1.0000
OB	98.72	99.04	97.27	99.55	0.96	1.28	98.96	0.9729	0.1671

DI - detection item, PPV - positive predictive value, NPV - negative predictive value, FP - false positive, FN - false negative, CR - coincidence rate, OB - occult blood, p-value is obtained by McNeymar's test.

batch and inter-batch precision of red blood cells were evaluated. The four columns of statistics data are shown in Table 4.

Linear analysis of RBC and WBC: High-concentration red and white cells were prepared, and then diluted by doubling ratios. The dilution ratio was taken as the abscissa and the cell concentration as the ordinate, and the regression equation was constructed to evaluate the linearity as shown in Table 5.

Accuracy assessment: The manual method and the instrument method were compared, and four sets of data were obtained, including both positives, both negatives, the manual method negative but the instrument method positive, and the manual method positive but the instrument method negative, as shown in Table 6.

Statistics of various parameters of instrument method: Using the data obtained in Table 6, the sensitivity, specificity, coincidence rate, and other relevant data about

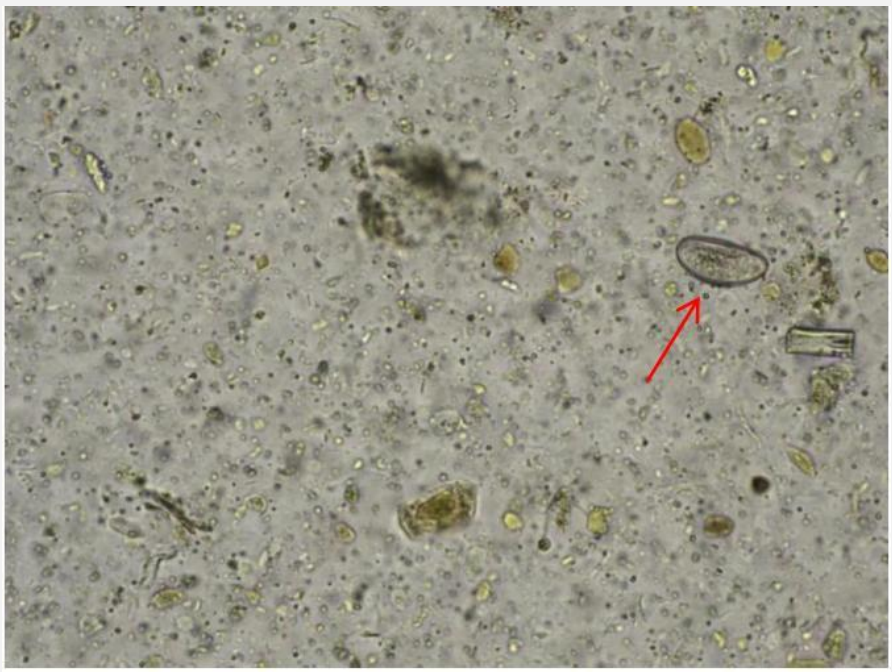

Figure 1. Pinworm eggs (10 × 20 times).

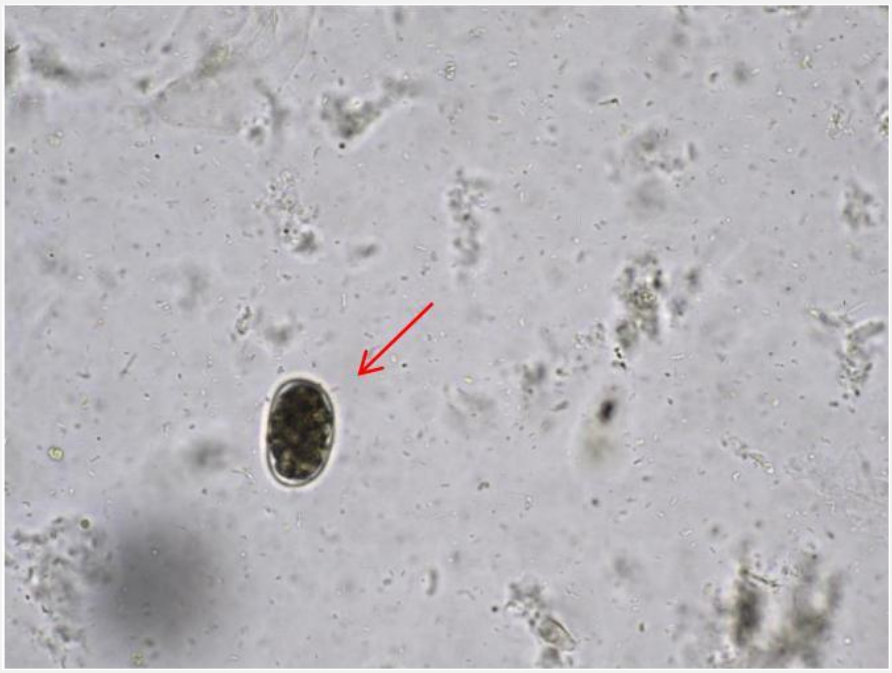

Figure 2. Hookworm eggs (10 × 20 times).

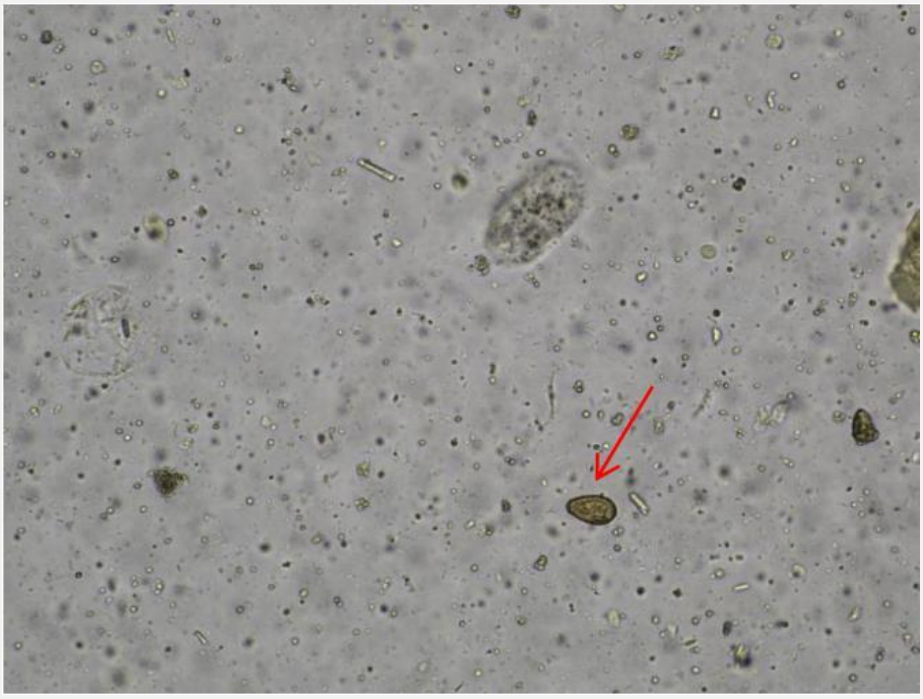

Figure 3. Clonorchis sinensis eggs (10 × 20 times).

RBC, WBC, fungus and occult blood were calculated, as shown in Table 7.

Pictures of parasites: 5 parasite specimens and 1 quality control product were collected during the work, which were tested and photographed on the instrument. The captured pictures are shown in Figure 1 - 6 below.

DISCUSSION

As the product of the human digestive system, feces can better reflect the state of the digestive tract, which is of great significance for clinical diagnosis and differential diagnosis. The traditional stool examination using a manual microscope has its own shortcomings: 1. Due to the different techniques of each person, the repetition between people is poor. 2. The odor and character of feces will affect the emotion of inspectors. 3. The quantity of specimens per unit time is too large and will affect the subjective judgment of inspectors. 4. The cover needs to be opened during the sampling process, and this open operation increases the risk of biosecurity. In this context, the automation of stool analyzer has been developed at a high speed (for example, the stool analyzer in the field has been updated from KU-F10 to the fourth generation KU-F40), which makes up for the shortcomings of the traditional manual microscopy to a large extent and improves the work efficiency. Take a simple example, stool collector has a number of patents, so that we can complete the detection without opening the collector lid, which meets the requirements of biosafety.

According to the industry standard requirements of Automatic Feces Analyzer, the detection rate of 10/μL cells is required to be no less than 90%. From the data in Table 2, the instrument meets the requirements in this point. Combined with our understanding of the detection limit of EP17-A2, the 10/μL here actually requires that the detection limit of existing fecal analyzers should be at least 10/μL, because the industry standard clearly sets the detection limit requirements. Therefore, only detection limit verification was carried out here instead of determining the cell detection limit of the analyzer not according to the requirements of EP17-A2.

In the assessment of the carry pollution rate, the carry pollution rate of RBC was 0% and that of WBC was 0%, which both met the requirement of 0.05% stipulated in the national standard. The specific data are shown in Table 3. A good carry pollution rate reflects the cleaning and decontamination capacity of the instrument to provide a guarantee for accurate results.

In the process of cell precision verification, CV value of low RBC (50 - 200/μL) in intra-batch precision was 14.4%, CV value of high RBC (> 200/μL) was 11.4%,

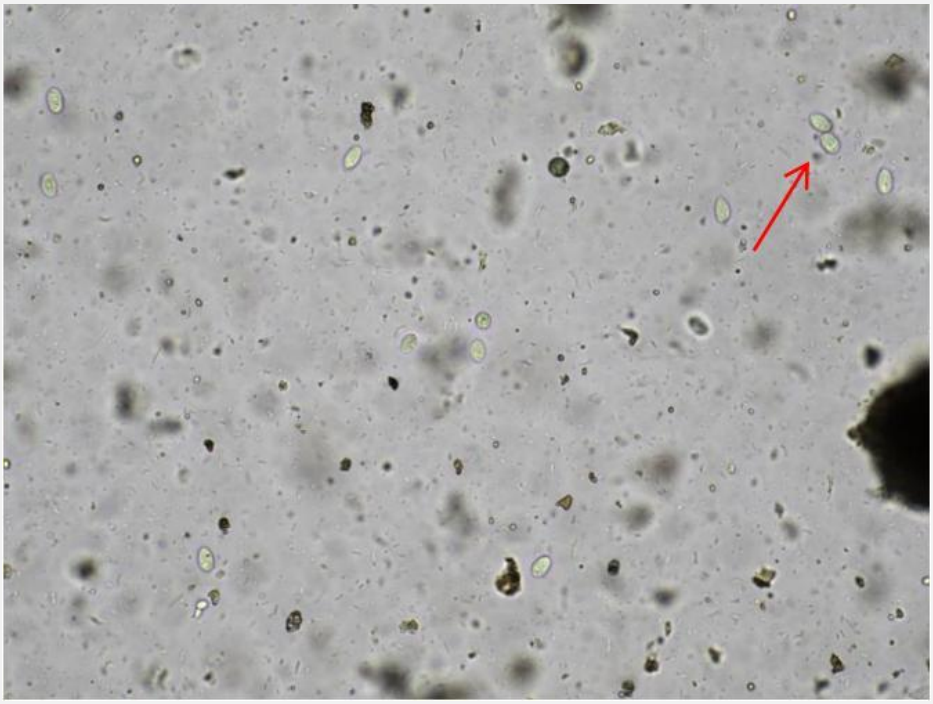

Figure 4 - 1. Giardia Lamblia cysts (10 × 20 times).

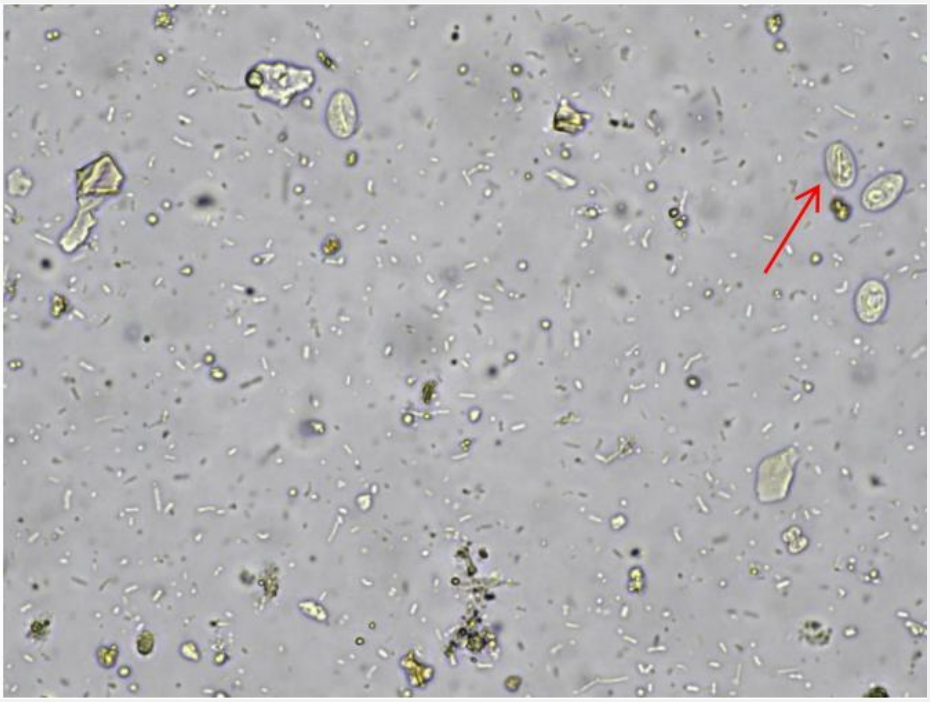

Figure 4 - 2. Giardia Lamblia cysts (10 × 40 times).

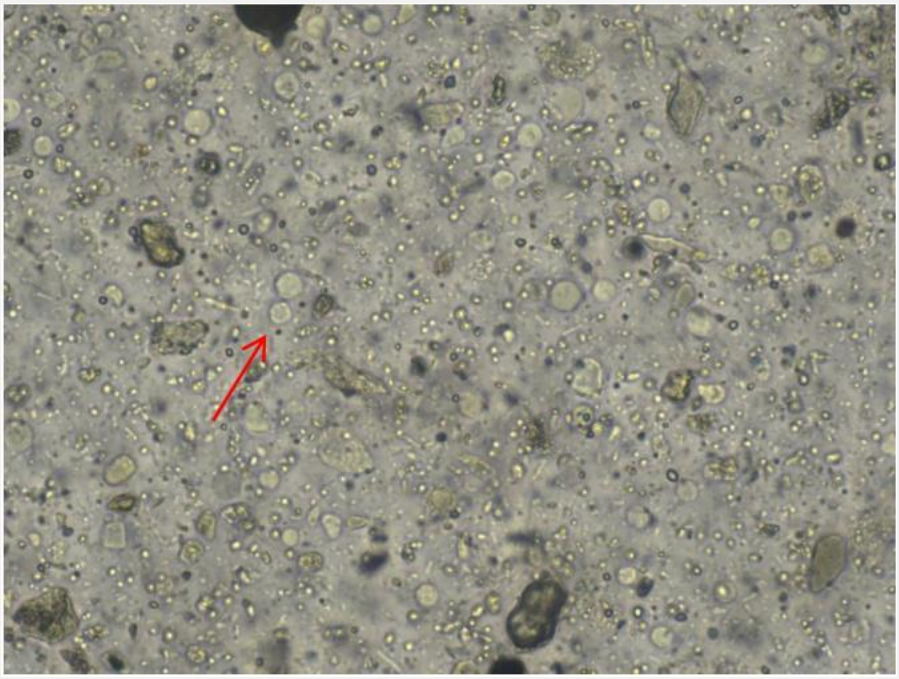

Figure 5. Blastocystis hominis (10 × 40 times).

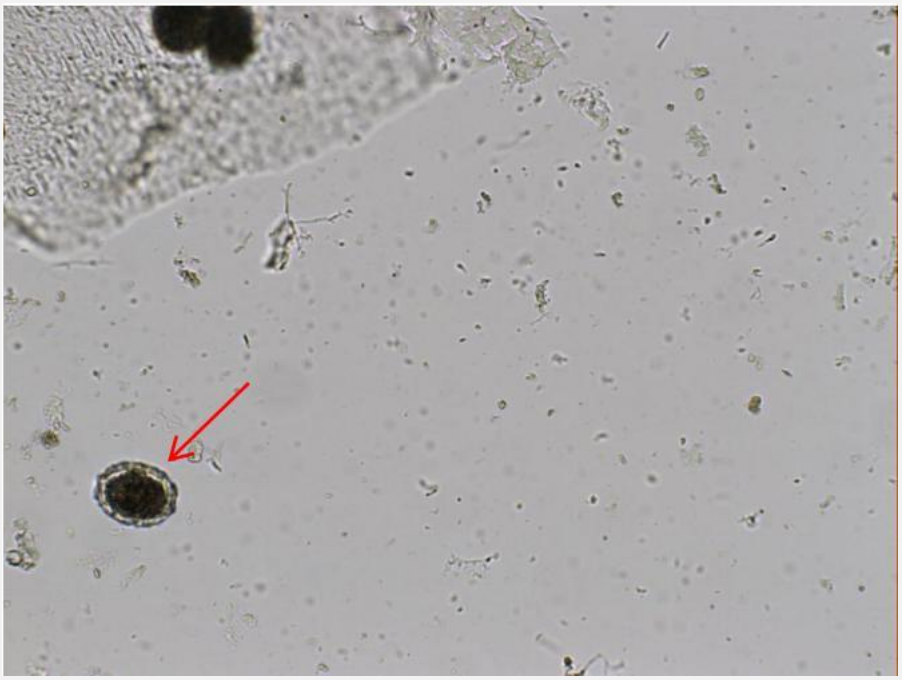

Figure 6. Ascaris eggs (10 x 20 times).

CV value of low RBC (50 - 200/μL) in inter-batch precision was 17.81%, and CV value of high RBC (> 200/μL) was 11.87%, which met the industry standard requirements of Automatic Stool Analyzer. The specific data are shown in Table 4. It should be noted here that the evaluation method of repeatability of visible components is slightly different from that of the EP15-A2 document. On the one hand, this experiment combines industry standards and on the other hand, it combines the daily work. The deficiency is that the quantitative monitoring of cells by the quality control products supported by this device is limited to RBC, with two levels of high and low value. There is only one detection limit level for WBC. Therefore, only the inter-batch precision of RBC was evaluated here, but the CV value of red blood cells could also reflect the imprecision level of particle determination by this device.

In the process of linear evaluation of red and white cells, a good correlation was obtained, and the R-value of red blood cells was 0.9990 and that of white blood cells was 0.9983. Specific data were shown in Table 5. A good linear correlation reflects the reliability of the results in the analytical measurement range and indicates that the device has quantitative detection as its core, although the reporting mode is the range mode.

When evaluating the accuracy of the equipment, the manual method is taken as the gold standard, and the instrumental method is compared with the manual method, so as to obtain the sensitivity, specificity, positive predictive value, negative predictive value, false positive, false negative, and coincidence rate of each item. The erythrocytes were 95.08%, 99.38%, 84.06%, 99.83%, 0.62%, 4.92%, 99.23%; The leukocytes were 92.68%, 99.83%, 96.20%, 99.66%, 0.17%, 7.32%, 99.51%; For fungi, 89.47%, 99.94%, 94.44%, 99.89%, 0.06%, 10.53%, 99.84%; Occult blood was 98.72%, 99.04%, 97.27%, 99.55%, 0.96%, 1.28%, 98.96%; The specific data are shown in Table 6 and Table 7. It can be seen that the instrumental method and the manual method achieve good consistency under the premise of setting in Table 1. Since normal and abnormal are not easy to define, physiological traits were not evaluated in this study. According to the National Guidelines for Clinical Laboratory Procedures, brown or yellow-soft physiological traits are considered normal. But in practice, the normal physiological traits fluctuate. For example, it is normal for newborns to have dark green stool, and it is normal for babies to have egg patterns from drinking milk. Therefore, physical characteristics should be evaluated according to individual differences.

In the process of parasitic eggs assessment, since there are relatively few cases of eggs and it is difficult to obtain them in a short time, only parasite cases retained in daily work can be detected and photographed to response their ability to capture eggs. A total of 6 kinds of parasites, 4 kinds of eggs and 2 kinds of protozoa, were photographed this time, as shown in Figure 1 - 6. Although there are various detection methods for parasites at present [8-11], the advantage of microscopic examination is that it is simple and intuitive and gives accurate clinical information. Figure 1 shows a pinworm egg collected in a pediatric clinic. Figure 2 is a hookworm egg from a 57-year-old man with colon cancer. Figure 3 shows a clonorchis sinensis egg found in a patient diagnosed with clonorchiasis. Figure 4 shows Giardia Lamblia cysts in the feces of a 27-year-old male patient with a renal tumor (Figure 4 - 1 for low magnification and Figure 4 - 2 for high magnification). Low magnification was used for capture and high magnification was used for identification. Figure 5 shows Blastocystis hominis. Its cell bodies vary in size. Compared with RBC, it has a biased nucleus. Compared with WBC (pyocytes), its cell bodies do not have the sense of white blood cells' fine particles, so its refractivity is stronger than that of WBC. Figure 6 is an ascaris egg photographed in the quality control product. When used, you can improve the ability to capture parasites by modifying the Settings in Table 1 (such as increasing the number of pictures taken, etc.) and at the same time conduct manual checks to prevent missed detection of parasites. As for how to set the parameters to make the equipment achieve the best detection limit for different eggs and protozoa, due to the limited specimens, this needs further study in follow-up research.

The instrument's 6 colloidal gold cassettes can also complete the test for transferrin, rotavirus, adenovirus, helicobacter pylori, calprotectin, which can provide a more powerful basis for clinically accurate diagnosis and effective treatment of digestive tract diseases.

From the performance of the instrument, the detection limit, precision, carry pollution rate, linearity, and so on meet the access requirements of the instrument into the laboratory. From the point of view of the instrument's compliance with system documents, this equipment has its own supporting quality control products, which solves the requirement of full coverage of indoor quality control required by ISO15189 system. In the future, the requirements for the introduction of equipment will not simply focus on the performance of the equipment, but the introduction of the whole detection system meeting the current quality standards. This also applies to the feces analyzer; the feces analyzer will be more and more in line with the clinical detection requirements under the continuous development in the future.

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Hongbo Zhang and Guo Li contributed to the intellectual content of this paper and drafted and revised the manuscript. Hui Zhang and Ming Huang contributed to the collection and analysis of the original experiment data. Chi Zhang and Yang Fei give some instructive suggestions to this article. All authors have read and accepted the final version of the manuscript and approved its submission to this journal.

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Ethics Approval:
The study protocol was approved by the Tongji Medical College Ethics Committee for Research in Health at Tongji Hospital and Complied with all relevant national regulations and institutional policies.

Declaration of Interest:
The funding organization(s) played no role in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the report for publication.

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