Performance Report

anvajo vet fluidlab 1

Background

Microscopic examination of urine is one of the most often performed and valued POC-tests in veterinary diagnostics. It is carried out to *screen* for and monitor diseases and conditions such as urinary tract infections or kidney disorders within animals. As automated techniques are very expensive for primary care usage and manual measurements can lead to inaccurate results, veterinarians struggle with the drawback of unstandardized analysis.

With its state-of the art quantitative phase imaging technique the anvajo vet fluidlab 1 allows automated urine microscopy of uncentrifuged urine and can thus enable faster diagnoses through the POC methodology and the elimination of unnecessary intermediate steps in sample preparation.

The anvajo vet fluidlab 1 is *the smallest urine microscope unit* globally available and is intended to be used for direct measurement of animal urine from cystocentesis, catheterisation or free catch.

The user aspires *20 µL of fresh*, *well-mixed urine* and fills the pre-manufactured microscopy slide. Then, the slide is inserted into the device and analysed immediately. anvajo vet fluidlab 1 works on the principle of Digital Holographic Microscopy (DHM) with automated particle recognition using convolutional neural network (CNN) combined with a MOPF (Multiple Object Plane Fusion) technology. The MOPF merges multiple focus planes and thus allows detection of objects in different layers and at the same time eliminates an incidence of out of focus images.

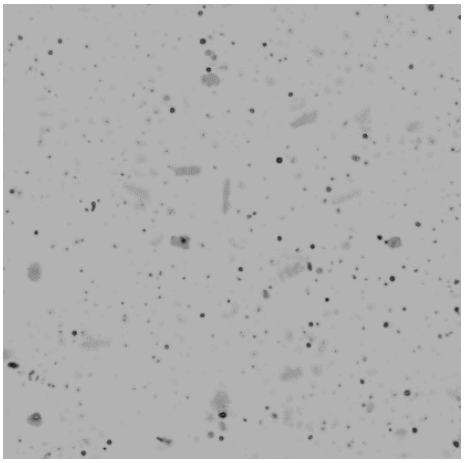


Figure 1. Example of reconstructed holographic microscope image, containing RBCs, WBCs, EC and casts.

Material and Methods

Specificity and Sensitivity

To analyse the specificity and sensitivity of the fluidlab 1, a total of 306 samples were measured and compared with reference measurements of the iRICELL® 2000 Plus (Beckmann Coulter) using Digital Flow Morphology method. The measurements were performed at the AMEOS clinic in Bernburg, Germany for anvajo.
A sample was classified as positive or negative depending on the listed threshold values (*Table 1*). A sample is positive if it exceeds the specified threshold. Red blood cells (RBCs), white blood cells (WBCs), and epithelial cells, divided into squamous (SEC) and non-squamous (nSEC) epithelial cells, were measured. The results of the comparison can be found in *Table 2*.

	Reference cutoff	anvajo cutoff
	N/µL	
RBC	100	69
WBCs	100	247
nSEC + SEC	100	96
SEC	20	21

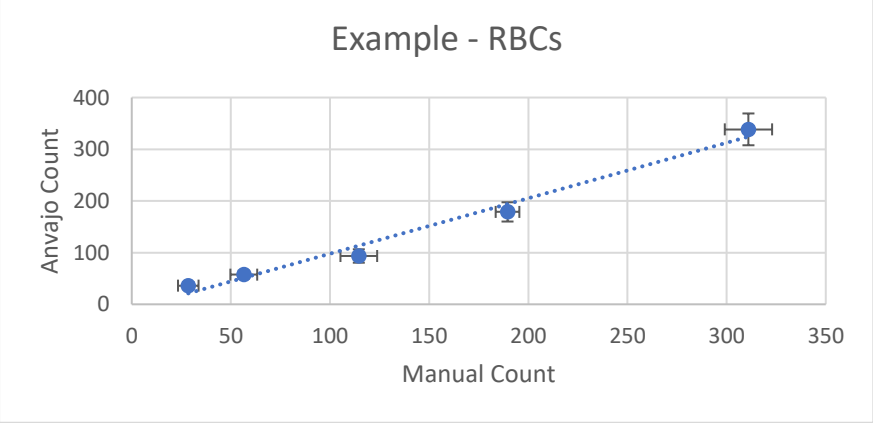
Table 1. Threshold values of the measured sensitivity and specificity.

	Sensitivity	Specificity
WBCs	91.8%	91.9%
RBCs	71.1%	71.3%
nSEC + SEC	93.3%	91.1%
SEC	84.6%	85.0%

Table 2. Specificity and sensitivity of the vet fluidlab 1 compared to counting of the iRICELL.

Reproducibility and Linearity

To determine the accuracy of measurements over multiple measurement points and different concentration ranges, RBCs and WBCs from synthetic urine were measured over 5 concentration ranges. Each measurement was performed five times on three different devices, resulting in CVs. Linearity between the different methods was compared with manual counting performed by experienced laboratory assistant with a microscope using the Neubauer counting chamber.
The reproducibility and linearity results are shown in *Table 3* and *Graph 1*, respectively.



Graph 1: Displays measurement linearity between anvajo vet fluidlab 1 and reference method.

	vet fluidlab 1	Manual count
	CV%	CV%
RBCs Dilution 4:1	10%	16%
RBCs Dilution 2:1	12%	10%
RBCs Dilution 1:1 (no dilution)	15%	6%
RBCs Dilution 1:2	21%	29%
RBCs Dilution 1:4	23%	23%
Linearity [R^2]	0,9948	

	vet fluidlab 1	Manual count
	CV%	CV%
WBCs Dilution 4:1	8%	7%
WBCs Dilution 2:1	15%	22%
WBCs Dilution 1:1 (no dilution)	13%	18%
WBCs Dilution 1:2	24%	6%
WBCs Dilution 1:4	27%	55%
Linearity [R^2]	0,9989	

Table 3. Measurement of different urine concentrations in 5-fold determination with three instruments to check the reproducibility and linearity of the measurements with the vet fluidlab 1.

Bacteria Flag

anvajo vet fluidlab 1 flags suspected presence of bacteria in samples. The flagging is issued as a function of WBCs concentration. In order to determine specific clinical thresholds as well as the sensitivity and specificity of the bacterial flag, a comparative study was conducted at the Veterinary University in Munich, Germany.

Bacterial Flag	
Sensitivity	78%
Specificity	80%

Table 4. Sensitivity/specificity of bacterial flagging compared to reference methods.

In total, samples from 179 patients were manually assessed through microscopic examination by experienced laboratory assistant. Further bacteriological examination was then issued for all samples showing the suspected presence of bacteria within the microscopy assessment. A total of 87 patient samples were additionally tested for confirmation of bacteria presence.
The results of the specificity and sensitivity of vet fluidlab 1 compared to bacteriological examination can be found in *Table 4*.

Measuring Time

Fast, but also precise and reliable measurement results are essential for the use of automated counting systems. Depending on the concentration of the sample the anvajo vet fluidlab 1 enables the automatic urine microscopy to be performed within 4 - 8 minutes.
Table 5 shows an analysis time with the vet fluidlab 1 in dependency on the sample concentration.

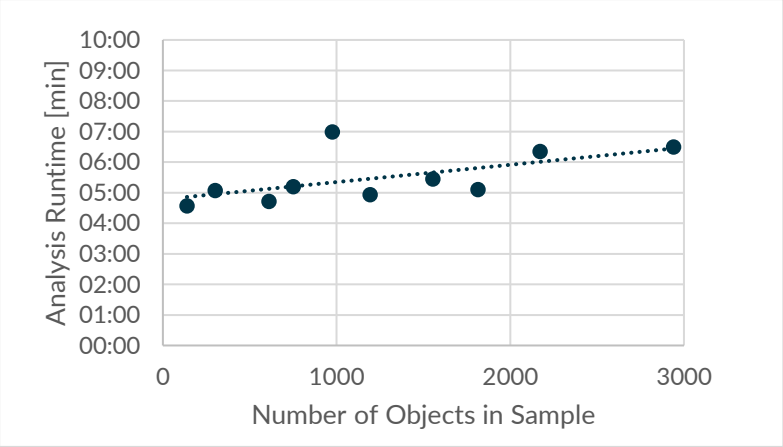


Table 5. Measurement runtime in relation to number of objects in the sample.

Discussion

The results of the performance study show that the anvajo vet fluidlab 1 provides precise and reproducible results compared to conventional urine microscopy methods. Although trained laboratory personnel are experienced in counting urine components, inaccuracies can still creep into the counts due to human error. Automated counting with the anvajo vet fluidlab 1 reduces the standard deviations of the counts, which can be seen well in *Table 3*. At the same time, the anvajo vet fluidlab 1 can clearly compete with established laboratory automated counters in terms of accuracy of results, as shown by the high sensitivity and specificity values in *Table 2*.

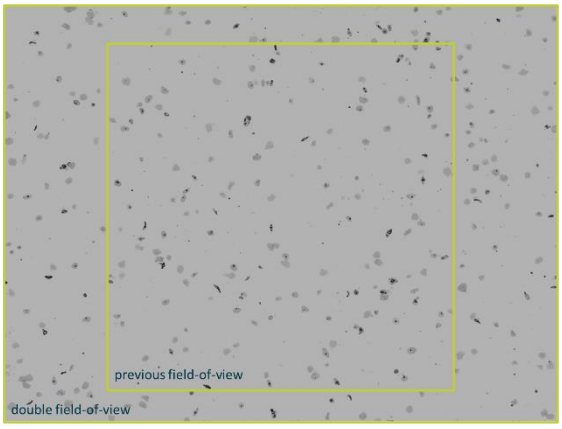


Figure 2. The new anvajo vet fluidlab 1 delivers an even larger field-of-view. The counted volume has doubled (>1µL). Thus, a high statistical accuracy is guaranteed.

The comparison of a Digital Flow Morphology method to a count using DHM, could explain the low values of specificity and sensitivity of the RBCs. Flow systems exert mechanical stress on cells that are already unstable due to the urine milieu, which may cause the absolute numbers of both measurement methods to differ.

In the results of the detection of bacterial contamination of the urine, it can also be seen that the anvajo vet fluidlab 1 provides a high measurement accuracy. The initial assessment by microscopic examination and the subsequent cultivation of the samples are very time-consuming and labour-intensive. For an initial assessment, the anvajo vet fluidlab 1 can therefore be well integrated into the laboratory workflow, as the measurement time of 4 – 8 min (*Table 5*) for a sample results in extreme time savings.