Exigo™ H400 analyzer design for optimized system performance

Exigo H400 hematology system is purpose-built to fit the needs of various-sized veterinary hospitals or smaller clinics or laboratories. The analyzer comes equipped with many features that help keep service and maintenance needs to a minimum as well as the performance and accuracy high. To ensure a reliable and long-term performance, however, some user intervention is still required, and adherence to determined service schedules is recommended. This document highlights instrument components that are critical for an accurate and reliable performance of the Exigo H400 analyzer, and maintenance requirements for the instrument and individual components are discussed.

Introduction

Complete blood count

A complete blood count (CBC) is the most requested analysis by veterinarians to assess and monitor patients' health condition. For this, automated hematology analyzers are frequently used in clinical laboratories to measure the oxygen-carrying red blood cells (RBC), the platelets (PLT) that help clot the blood, and the white blood cells (WBC) of the immune system.

As part of the CBC, a differentiation of the WBCs into their subgroups is typically conducted. In normal mammalian blood, these subgroups are usually divided into five major categories:

- The neutrophils (NEU) account for about 60% of the WBCs. NEUs are the first line of defense and help fight mainly bacteria (and fungi) through phagocytosis, and a high count (> 85%) can therefore indicate a bacterial infection.
- Lymphocytes (LYM), accounting for about 30% of all WBC, are part of the adaptive immune system and mainly help fight viruses. A high LYM count can therefore be an indication of a viral infection. On the other hand, a low LYM count with a slight MONO elevation is a common finding in especially cats due to stress
- The last 10% comprises monocytes (MONO), eosinophils (EOS), and basophils (BASO). These cell types are typically associated with allergies or parasite infections. A high MONO count, for example, can indicate a chronic inflammatory disease, whereas high EOS counts give an indication of asthma, an allergic reaction, or a parasite infection. A high BASO count is typically associated with inflammatory reactions, especially those causing allergic symptoms.

High numbers of the WBCs can also be an indication of certain forms of cancers, such as leukemia or lymphoma.

In-house point-of-care diagnostics

The reason for having in-house hematology diagnostics is to aid in the diagnosis by, for instance, providing quicker laboratory results to the clinic or hospital. If the results are not reliable, it would be preferable for the lab to send the blood samples to a reference laboratory. Performance varies largely between veterinary hematology analyzers. Apart from the main reason being design issues, another reason is that the instruments are in most cases not governmentally regulated ensuring they meet their stated claims.

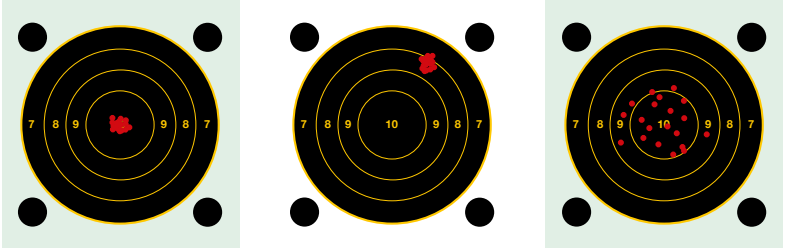
With a poor reproducibility, accuracy cannot be achieved. To compare the accuracy of two different hematology analyzers, satisfactory precision should first be ensured. The comparative accuracy study should include both normal and abnormal samples for the test and reference analyzers in a controlled environment under the same condition. With abnormal samples, accuracy is even more critical.

The Exigo H400 is designed to be a robust, easy to use and high-quality instrument and prides itself in its extremely high precision and accuracy (Table 1). The analyzer is especially tailored for clinics with small animals, dehydrated animals or with body fluid with some patented special features.

The high accuracy of the instrument is due to the measuring techniques, system components and reagents of the system, that will be explained in more detail throughout this document. The Boule vet calibrator is available to adjust and measure the accuracy of the system and the Boule vet control to check the precision and address random errors (Figure 1).

Table 1. CV% repeatability is measured as an average of 10 measurements each on 9 different dog venous blood samples in K_2-EDTA collected normal samples, on three instruments, in open tube mode

	RBC	MCV	HCT	PLT	MPV	HGB	WBC
CV%	0,8	0,3	0,9	4,6	2,7	0,9	3,5



Accuracy	Yes	No	Yes	No
Precision	Yes	Yes	No	No

Figure 1. A calibrator is used to measure and adjust systematic error to ensure the instrument is working accurately, while a control is used to address random error and indicate whether the instrument works precisely.

Measurement technologies

Impedance is commonly used for the RBC, PLT, and WBC counts, and for the differentiation of the WBCs into LYM, granulocytes (GRA) and MID cells (Figure 2). The LYM region constitutes mainly of lymphocytes. Other cells that might reside in this region include nucleated red blood cells, clumped platelets, macrocyte platelets, variant (atypical) lymphocytes, or blasts. The MID-cell area consists mainly of MONOs but can also correlate to degranulated NEU, precursor cells, blasts and plasmacytes. The GRA region mainly comprises NEU but can also include EOS and BASO (Figure 3).

Being able to distinguish between eosinophils and basophils from neutrophils can provide a more detailed and targeted assessment of the blood status. Instruments reporting eosinophils and basophiles usually require more advanced technologies and therefore come with increased costs and complexity, often requiring more maintenance (Reference 1). For the veterinary clinics, eosinophil counts are generally requested as it gives more detailed information regarding allergies and parasite infections, which is common among veterinary patients. However, the clinical significance of basophiles still remains largely unknown. Moreover, the fact that basophiles are very rare, together with the extended inter- and intra-species variation among them, makes it very difficult for automated hematology analyzers to detect and report precise absolute counts.

Exigo H400 has therefore been developed with the patients and diagnostics in focus, where the eosinophil count is present at a lower complexity and cost through impedance, yet without compromising the accuracy of the results. The basophil count has been disregarded on the H400 due to its low abundance and the low accuracy the results the analyzer would have given the user. This means that the Exigo H400 is considered a 4-part differential hematology instrument.

For general screenings, hematology analyses based on impedance technology will provide sufficient information. A simple CBC will help detect anemia and blood clotting problems and answer the question of a viral infection or a bacterial infection that can be treated with antibiotics. In addition to a CBC, an analyzer also determines a range of other parameters such as hematocrit (HCT), the mean corpuscular volume (MCV), RBC distribution width (RDW), mean PLT volume (MPV), and PLT distribution width (PDW). It also measures. the oxygen-containing hemoglobin (HGB). HGB measurements are commonly conducted by photometry. This, together with a 4-part differential of the white blood cells, means the Exigo H400 will provides the user with more detailed hematological overview.

No matter the hematology analyzer in use, if abnormal values are obtained, microscopy is recommended. Although 3-part differentials provide excellent precision for general screenings, accuracy is improved with 4-part differentials for abnormal samples, reducing the number of manual blood smears.

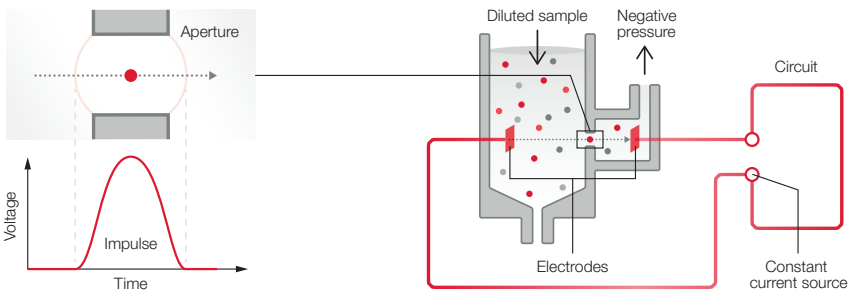


Figure 2. The principle for measuring changes in the electrical impedance produced by a cell passing through an aperture. Each cell passing through the aperture causes a drop in the electrical current (a pulse). The number of generated pulses correlates with the number of cells, whereas the size of the pulse is related to the cell size.

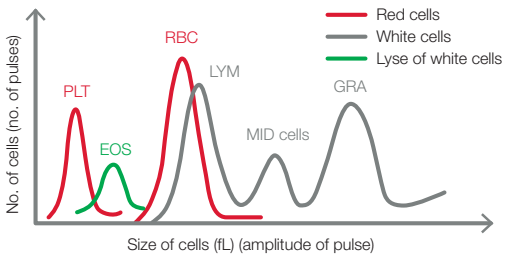


Figure 3. Hematology analysis results are visualized in histograms.

Exigo H400 - innovation built on total quality

Exigo H400 is tailored to be adjustable to various-sized laboratories and veterinary hospitals (Figure 4). The analyzer features a high-precision shear valve for accurate sample aspiration and dilution. A closed shear valve design minimizes leakage risk, ultimately reducing the maintenance requirements, yet up-holding the high sample volume accuracy for a high quality result. Exigo H400 employs well-proven and robust measurement technologies. The analyzer uses impedance for WBC, RBC, and PLT counts, while HGB is determined spectrophotometrically. The analyzer provides quantitative results for 19 parameters, with histograms for WBC, EOS, RBC, and PLT (Figure 5).

The sample analysis software displays intelligent information messages related to both instrument flagging as well as sample pathology messages. The sample pathology messages define the sample abnormality followed by recommendations for further investigation of that sample. The instrument flagging gives software and system information messages regarding abnormalities in the sampling and analysis that occur.

The information can be triggered by the following mechanisms:

- Histogram-shape abnormalities detected by system software calculations.
- Selected values that exceed defined limits outside the reference range. These messages occur when selected values are moderately to markedly abnormal. Values slightly outside the reference range are typically treated as cautionary by the clinician.

Figure 4. Exigo H400 system with built-in tube mixer and Micro-pipette adaptor (MPA).

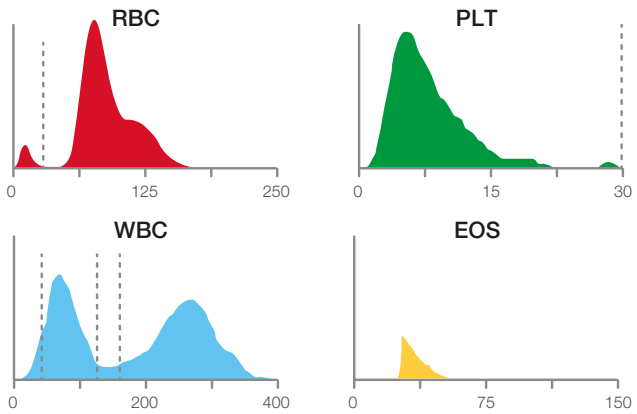


Figure 5. Exigo H400 analysis results visualized in histograms for WBC, RBC, PLT, and EOS.

Exigo H400 provides a robust performance, with analysis results comparable with those from a more advanced reference instrument (Figures 6 to 8).

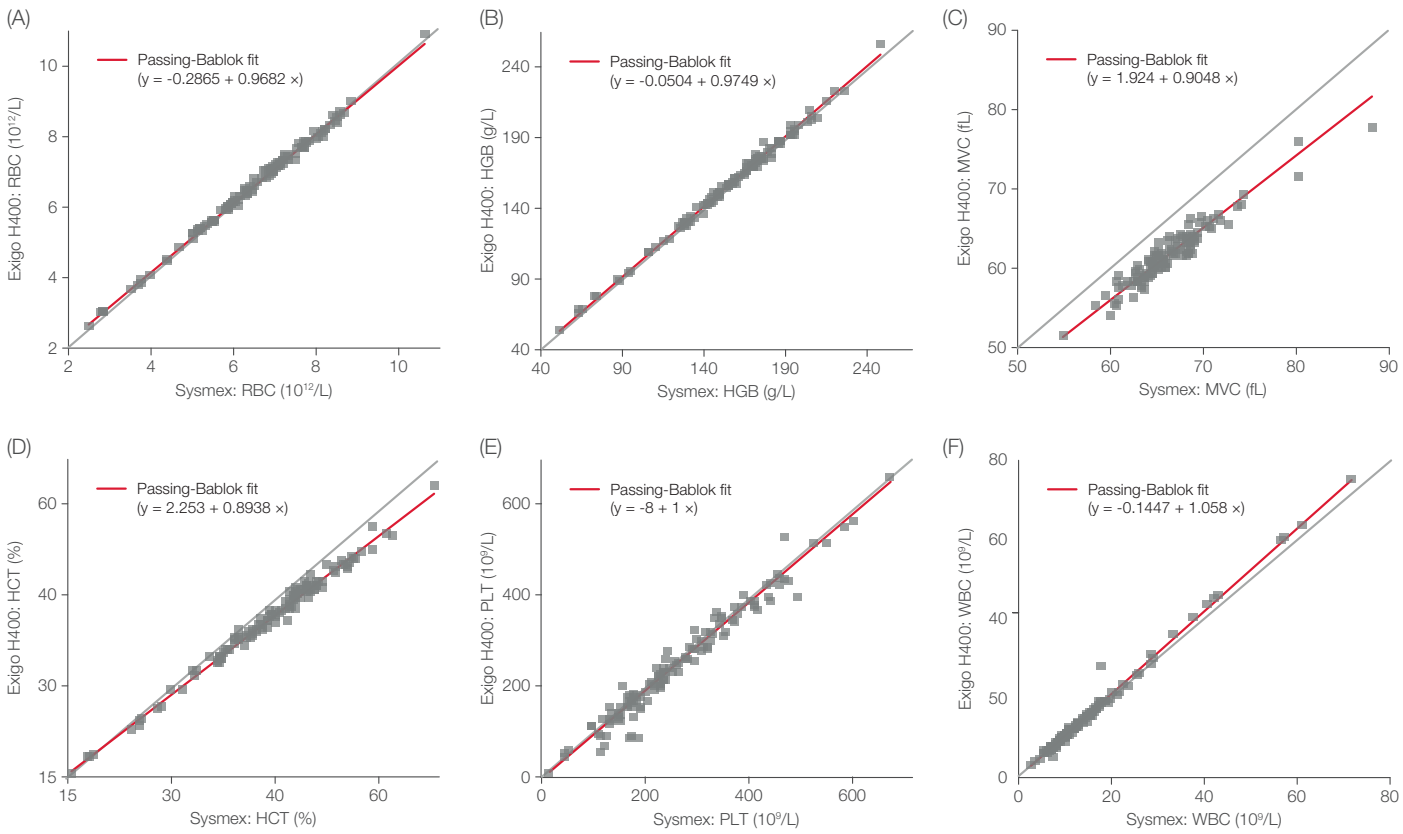


Figure 6. Agreement, using both unflagged and flagged dog samples, between Exigo H400 hematology system and a more advanced Sysmex™ XT-2000iV reference systems intended for the larger hospital laboratory. Correlation plots for (A) RBC, (B) HGB, (C) MCV, (D) HCT, (E) PLT, and (F) WBC. In the regression plots, the gray line corresponds to identity (x = y) and the red line corresponds to best fit.

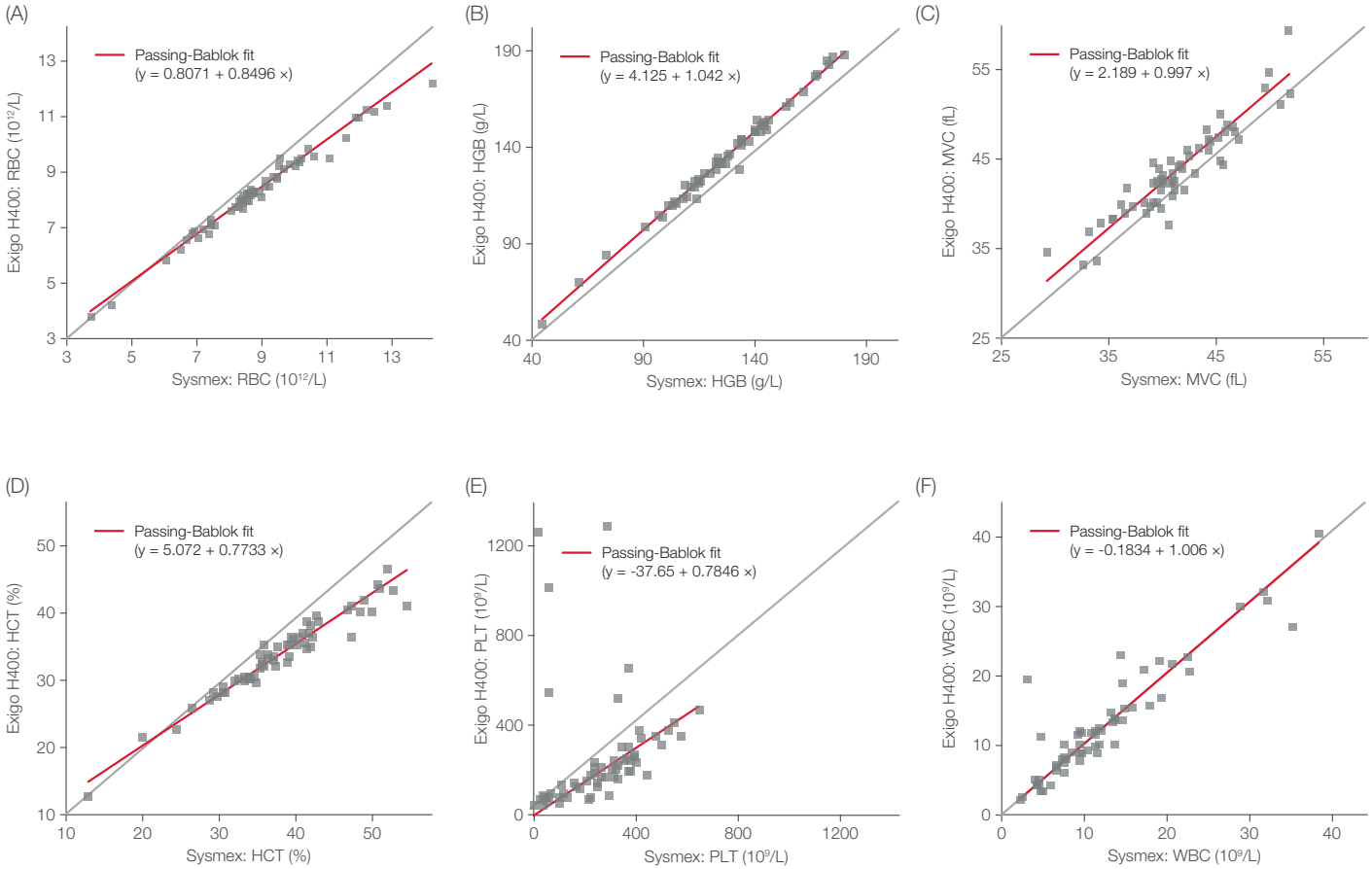


Figure 7. Agreement, using both unflagged and flagged cat samples, between Exigo H400 hematology system and a more advanced Sysmex XT-2000iV reference systems intended for the larger hospital laboratory. Correlation plots for (A) RBC, (B) HGB, (C) MCV, (D) HCT, (E) PLT, and (F) WBC. In the regression plots, the gray line corresponds to identity (x = y) and the red line corresponds to best fit.

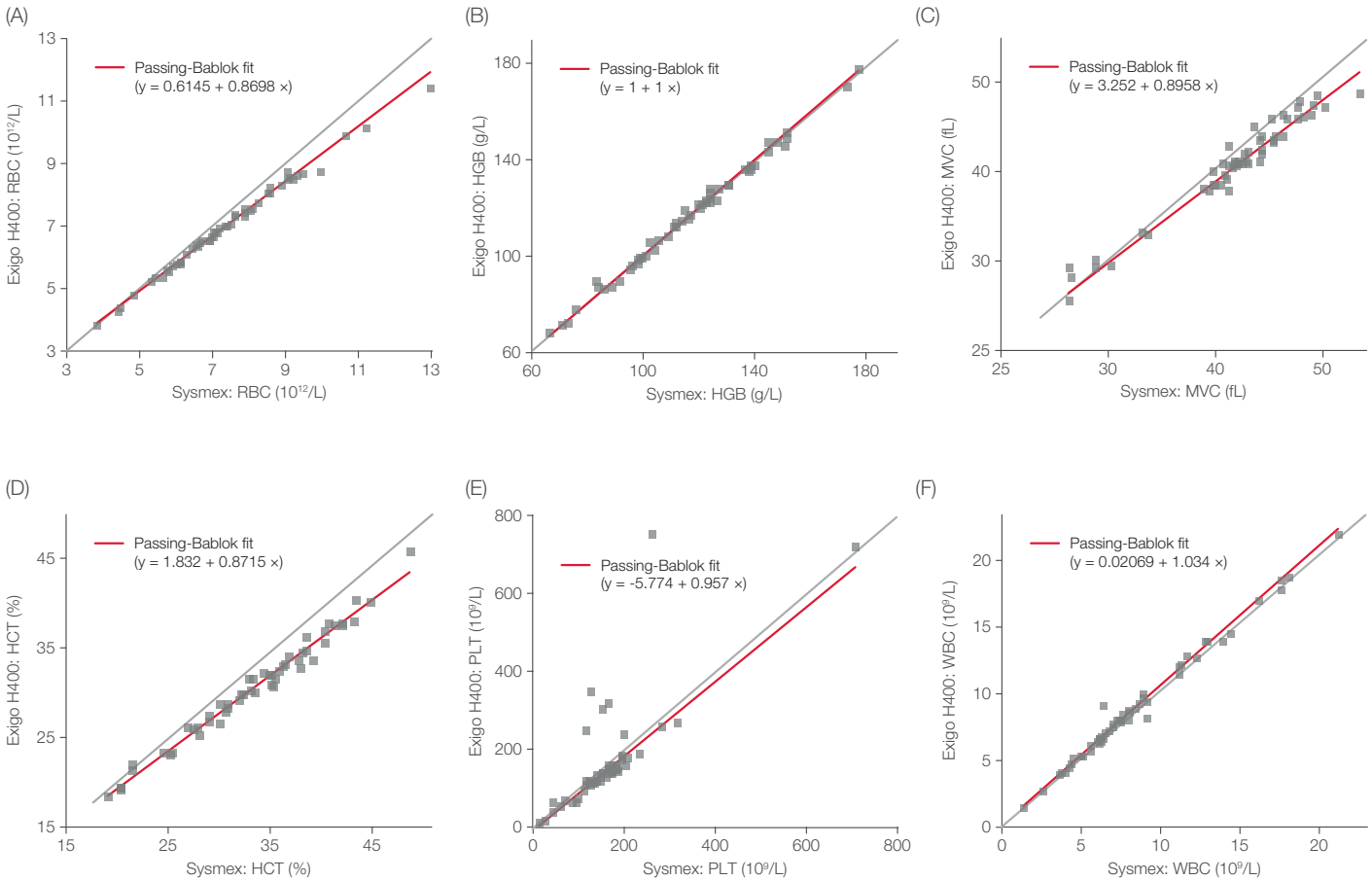


Figure 8. Agreement, using both unflagged and flagged horse samples, between Exigo H400 hematology system and a more advanced Sysmex XT-2000iV reference systems intended for the larger hospital laboratory. Correlation plots for (A) RBC, (B) HGB, (C) MCV, (D) HCT, (E) PLT, and (F) WBC. In the regression plots, the gray line corresponds to identity (x = y) and the red line corresponds to best fit.

System components

Sampling technologies

The sampling technique is critical for accurate and reliable analysis results. An exact volume of blood needs to be precisely diluted in a highly repeatable manner. Two different sampling techniques are commonly used in today's hematology analyzers: either a micro-pipette connected to a vacuum-generating, step motor-controlled syringe pump or a rotating shear valve. The suction process of the syringe pump is often time-based, and the technology is considered less complex and therefore a more cost-efficient solution. However, a syringe doser is more sensitive to variable pressure conditions and requires a mechanism for moving the sample probe in horizontal and vertical directions. This mechanism will involve more frequent monitoring of external conditions possibly affecting results, as well as more frequent service to replace worn-out moving parts.

In contrary, the rotating shear valve allows cutting an absolute volume for analysis, and is not sensitive to altitude and other factors that might affect pressor conditions. On the other hand, shear valve technology is considered more complex and therefore a more expensive solution. Many shear valves also have a design that makes them sensitive to environmental impurities that might cause leakage and thus will require regular cleaning.

Exigo H400 shear valve technology

Every Exigo H400 analyzer comes equipped with a high-precision shear valve that cuts out an absolute sample volume to be used for analysis (Figure 9). The closed design makes the shear valve maintenance-free, thereby lowering maintenance costs. Atmospheric pressure variations will not affect the blood cell count. High altitude compensation only needs to be activated if various indicators related to HGB measurement problems repeatedly appear (see Section 9 in User manual). At higher elevations, the mode might need to be changed to Moderate or Maximum compensation. For high altitude compensation, the software incorporates some minor timing sequences for the wash cycles, no other functions are affected.

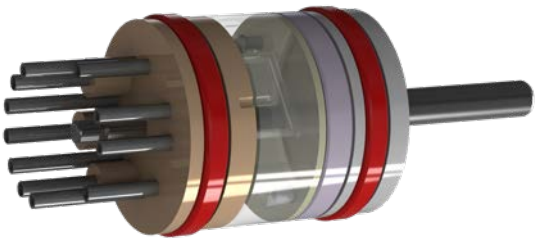


Figure 9. Maintenance-free Exigo H400 valve secures accurate results and lowers maintenance costs.

A blood sensor prevents inaccurate results caused by air in the sample. When enabled, aspiration stops when blood is detected by the blood detector sensor. This functionality can be disabled by the operator to instead employ a fixed aspiration type.

Sample aspiration modules

To maximize utilization of the Exigo H400 analyzer, sample aspiration can be performed through two different aspiration modes (Figure 10). The whole blood sample probe aspirates from open tube for analysis. After aspiration, the analyzer will perform an automatic probe flush for cleaning of the sample probe.

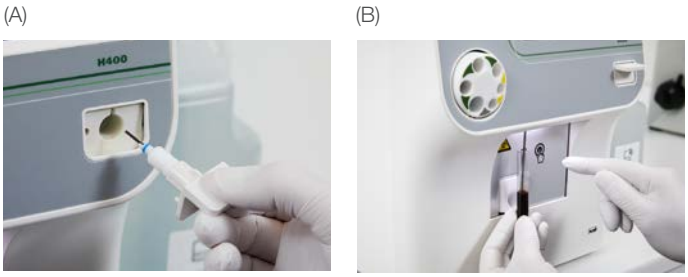


Figure 10. Exigo H400 allows sample aspiration from (A) open tube or (B) micro-pipette adapter.

Micro-pipette adapter (MPA) inlet

The micro-pipette adapter (MPA) enables CBC from one drop of blood using a capillary sample tube. The MPA functionality is well-suited for small animals, dehydrated patients or with body fluid samples. Only use Boule-supplied, plastic, high precision EDTA capillary tubes with the MPA inlet. Glass tubes can cause damage to the analyzer if inserted incorrectly.

As the MPA inlet bypasses the shear valve sample aspiration, it is of utmost importance to ensure correct volume is collected by making sure the whole capillary is filled with blood and by wiping of any excess blood outside of the capillary before sliding it into the MPA module (Figure 11).

Using the MPA also means cost-saving in terms of no needed vacuum tubes, microtainers or similar tubes. Moreover, no pre-mixing of the sample is required and you can also just sample a single drop of blood from for instance, the ear of a cat.

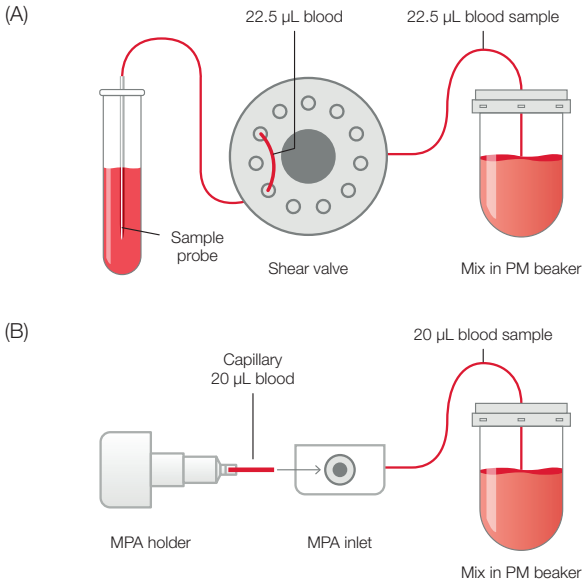


Figure 11. Sampling for (A) open tube inlet, using the shear valve, as well as for (B) the MPA inlet, bypassing the shear valve. The difference in sample volume is compensated for in the instrument software.

Measurement chambers

RBC and PLT counts are conducted in the RBC chamber, using floating discriminators. A typical challenge with animal blood is the small RBCs and PLTs of some animal species that are hard for automated hematology systems to detect. Due to these large variations in the size of RBCs and PLTs (MCV and MPV), Exigo H400 is equipped with a narrower capillary aperture of 60 µm, as compared to that of Boule's human analyzer utilizing a 80 µm aperture. This enables for smaller RBC and PLT cells to be detected in the capillary and with a higher sensitivity.

WBC count is conducted in the WBC chamber. As for RBC and PLT, the WBC differential is performed using floating discriminators to estimate the best separation between the cell populations (Figure 12).

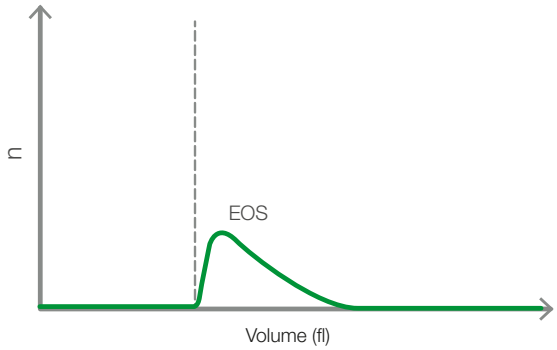


Figure 12. The Exigo H400 system uses a floating discriminator technology to estimate the best separation between three populations of white blood cells (LYM, GRA, and MONO cell fractions) and a fixed discriminator when calculating the EOS cell fraction.

The EOS count is also conducted in the WBC chamber and similar to the WBC, RBC and PLT count, where the eosinophil count is also measured using impedance technique together with fixed discriminators. After the WBC count is performed, new sample is obtained from the first aspiration by passing through the shear-valve to get an exact volume (Figure 13).

HGB is determined from the same dilution as the WBC (Figure 14). The HGB reading is slightly corrected for turbidity in case of extreme WBC counts. When the analyzer is in standby mode, the LED lamp is switched off to extend its lifetime.

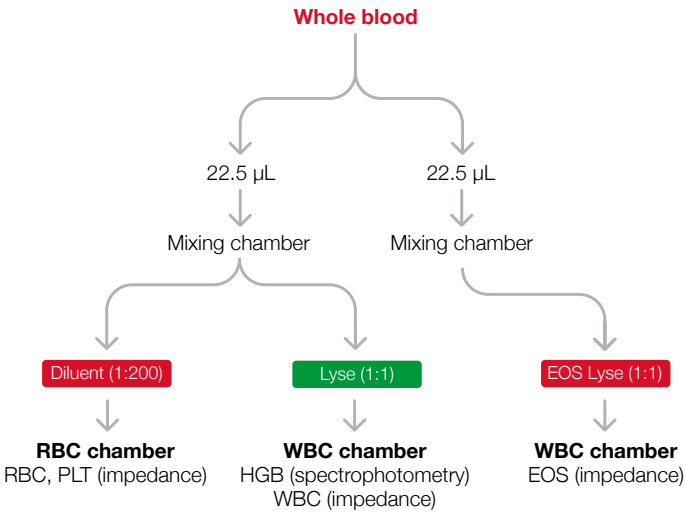


Figure 13. Exigo H400 measurement principle.

Liquid system

The fluidic system is controlled by pumps that generate pressure and vacuum. Reagent pipettes, featuring optical sensors, ensure accurate dilution of the sample. No pistons or other moving parts are used in the dilution system to minimize the maintenance and service needs. For the cell count, measuring pipettes equipped with liquid start and stop sensors ensure that a correct volume is used for analysis. The air pump generates a pressure that pushes the finally diluted sample through the aperture in the measuring chambers. To reduce risk for clogging, high voltage burning of the aperture is automatically carried out, but only when needed to reduce ware and tare.

To minimize user intervention, the analyzer performs automatic cleaning every 48 hours, using diluent. On the Exigo H400 system, there is an additional enzymatic cleaning cycle which uses the on-board cleaner reagent to dissolve proteins and for clot prevention. The on-board cleaner occurs at each enter-standby cycle. Boule-designed maintenance-free valves automatically relieve upon standby to prevent wearing of the tubing.

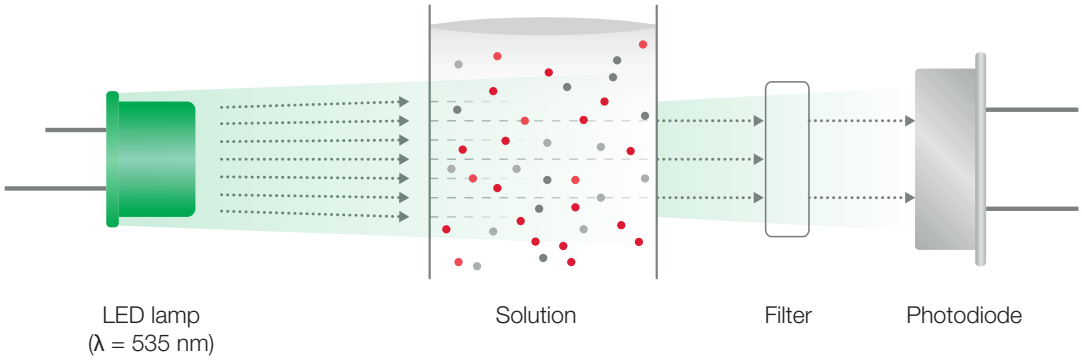


Figure 14. HGB is determined spectrophotometrically, using a LED lamp mounted on one side of the WBC chamber. The light is allowed to pass the flow chamber and transmitted light is detected by an optical sensor mounted on the opposite side. HGB concentration is calculated as a difference of a blank and a blood measure with and without illumination to reduce the effect of liquid refraction and disturbing light.

In the event of an error message, verify that the analyzer is filled and run a prime cycle, using built-in maintenance tools. The prime cycle is used to reset the analyzer after an error has been indicated or a failure in running a sample occurs.

Reagents

Depending on the analysis the user wishes to perform, two or three reagents are required for the Exigo H400 analyzer—Diluent and Lyse for a 3-part analysis (LYM, MONO and GRAN counts) or Diluent, Lyse and EOS Lyse for a 4-part analysis (LYM, MONO, NEU and EOS counts) — which greatly facilitates handling and logistics and helps reduce running costs. Moreover, on the Exigo H400 veterinary system, mainly due to the composition of animal blood, there is an additional Cleaner reagent, consumed only during enter standby. One of the main reasons why the Exigo H400 is extremely robust is due to this purpose-designed cleaner which allows the user to run body fluids, whilst eliminating all monthly cleaning procedures.

The reagents for the Exigo H400 are delivered with a reagent tray to make the bench space more organized. Simply scan the RFID card on the reagent container and the analyzer stores key information such as lot number, open and expiry dates, and remaining volume. The measurement principle is depicted in Figure 13. Not only is the blood dilution ratio critical for an accurate count, the reagent composition is also of utmost importance for reliable results. The Diluent should provide an isotonic environment for the RBCs and PLTs, while the Lyse reagent should be capable of lysing the RBCs to release HGB and shrink the WBCs to allow differentiation of these cells into their subgroups. The EOS Lyse then further lyses all the WBCs except for the more robust membranes of the eosinophil cells allowing the Exigo H400 to only detect and count them separately. Boule's cell count processes have been tested and optimized for decades for robust and reliable analysis results. The use of the reagents designed by Boule Diagnostics for the specific instrument ensures analytical quality and performance of the hematology system.

To ensure the dilution will always be exact and that there is no shortage of reagent, Boule adds a minimum volume to each reagent container and that should never utilized even after all cycles per reagent container are consumed to ensure no air will enter the system and compromise the accuracy of the results. For contamination purposes, this left-over reagent in the bottles after all cycles are consumed shall not be combined with newly opened reagent containers.

Instrument maintenance

Designed with few moving parts, a maintenance-free closed shear valve design, and with the majority of the instrument cleaning procedures being automated, the user maintenance of Exigo H400 analyzer is kept to a minimum. However, some user intervention is still required. Section 10 "Analyzer care and maintenance" in User manual contains information on how to maintain the Exigo H400 analyzer. An overview of maintenance procedures is given in Table 2.

Table 2. Scheduled maintenance

Procedure	Description	Frequency
Sample probe cleaning	Clean with paper tissue moistened with a 70% alcohol solution. Remove possible traces of salt crystals or blood at the top of the sample probe and probe rinse cup using a paper tissue moistened with the alcohol solution.	Daily
Surface cleaning	Gently clean the display and/or outside of the analyzer with a soft cloth, slightly moistened with water and a mild soap. Dry carefully.	When necessary
Clot prevention	Fill a small container with 5 mL of Enzymatic Cleaner from Boule Cleaning Kit. From ***Main Menu***, press ***Maintenance*** and then press ***Clot Prevention***. - Hold the cleaner container under the OT probe, submerged in cleaner, press OK to confirm. Perform a background check to verify that all values are within range.	Every 1000 samples
Cleaning procedure	Select ***Main Menu***, then ***Maintenance***, and arrow over to next page to enter the ***Cleaning Menu***. Follow instruction for use (IFU) for the Boule Cleaning Kit to clean the analyzer.	Less than 50 samples/day = every six months More than 50 samples/ day = every three months 100–200 samples/ day = every month.
Preventive maintenance (PM)	Inspection, and adjustments upon need, performed by an authorized service technician. PM kit available and included components should be exchange by an authorized service technician.	Every year or 20 000 samples

Good practice also dictates keeping the instrument clean from dust and other impurities. Regularly, check if there is dust inside the instrument. At the same time, check that reagent connection or waste tubes are not bent or squeezed. Also, regularly check for possible leakages from components inside the instrument.

The system software monitors a number of system functions and will display information that alerts the operator to check the system or sample, or institute selected troubleshooting procedures.

Quality control

Exigo H400 hematology analyzer is part of Boule's Total Quality Concept which is designed to increase the value of reported hematology results. Controls and calibrator are key elements of this initiative. Boule QC materials (Boule Con-Diff Vet and Boule Cal Vet) ensure that Exigo H400 performs accurately and delivers quality-controlled hematology results. Advanced quality control capabilities built into the Exigo H400 M32 software include Mean, SD, CV, Levey-Jennings charts, and QC reports.

The analyzer has been factory calibrated prior to shipment. If necessary, however, a calibration functionality is available. Good laboratory practice dictates regular checks and calibration of the measured parameters. Only authorized operators can update or change calibration factors.

Conclusion

Exigo H400 is an automated hematology system tailored to be adjustable to various-sized laboratories and veterinary hospitals, with patients of different animal species. Equipped with robust and well-proven technologies, the analyzer provides accurate and reliable analysis results that are comparable to those of a more advanced reference instrument intended for larger hospital laboratories. To maximize instrument uptime and ensure a reliable performance, adhering to determined maintenance procedures and service schedules is recommended. Following these guidelines, Exigo H400 will constitute a powerful tool that aids veterinarians in diagnosis and monitoring of disease progression and efficacy of treatment.

Reference

1. Whitepaper: Hematology analyzers: 3-part or 5-part, that is the question. Boule Diagnostics, 31183, Edition 1 (2019).
2. Gröndahl, G. Veterinary Hematology – An introduction. Boule Diagnostics, 33267, Edition 4 (2019).

Ordering information

Product	Product code
Exigo H400	1420001
Exigo Diluent 10 L, RFID	1504504
Exigo Diluent 1.9 L, RFID	1504501
Exigo Lyse 1.9 L, RFID	1504500
Exigo Cleaner 1.9 L, RFID	1504503
Exigo EOS Lyse 1.9 L, RFID	1504502
Boule Cleaning Kit, 3 × 450 mL	1504111
Boule Enzymatic Cleaner, 100 mL	1504112
Boule Hypochlorite 2.0% Cleaner, 500 mL	1504113
Boule Vet Con Normal, 1 × 4.5 mL	1504026
Boule Vet Con Normal, 6 × 4.5 mL	1504027
Boule Vet Cal 1 × 3.0 mL	1504028

Related literature	Product code
User Manual: Exigo H400	1504496
Quick Reference Guide Exigo H400	1504499

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