Research

Standard Operating Procedure for the Handling and Processing of Canine Blood Samples and Performance of Routine Hematological and Biochemical Analyses within the Nanostructurome Pipeline

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Abstract:
This Standard Operating Procedure (SOP) outlines the standardized collection, handling and processing of canine venous blood samples for analytical purposes using serum, plasma, or whole blood. It includes protocols for the preparation of serum and plasma, as well as the dilution of whole blood and plasma for the measurement and characterization of extracellular vesicles. The SOP also details the procedures for routine hematological and biochemical analyses, which are integral to the comprehensive evaluation of animals enrolled in the Nanostructurome project. By addressing the preanalytical phase and analytical performance, this SOP ensures consistency, minimizes variability, and reduces the risk of preanalytical and analytical errors. Its implementation is essential for generating accurate, reliable laboratory results, thereby supporting both high-quality research and effective clinical decision-making.

Keywords: Biochemistry; Blood samples processing; Hematology; Plasma; Preanalytical errors

Table of contents:

1. Definitions

ALT: Alanine aminotransferase
ALP: Alkaline phosphatase
BASO: Basophil
CBC: Complete Blood Count
Cl: Chloride
DIFF: White blood cell differential count
EDTA: Ethylenediaminetetraacetic acid
EVs: Extracellular vesicles
EWC: European Waste Code
HCT: hematocrit
HGB: Hemoglobin concentration
K: Potassium
Na: Sodium
NaCl: sodium chloride
PEROX: Peroxidase
PLT: Platelet count
PPE: Personal Protective Equipment
QC: Quality Control
RBC: Red Blood Cell count
WBC: White Blood Cell count
SOP: Standard Operating Procedure
MCV: Mean Corpuscular Volume
MCH: Mean Corpuscular Hemoglobin
MCHC: Mean Corpuscular Hemoglobin Concentration

2. Background

Providing high-quality care to animal patients is a fundamental goal of veterinary medicine and often relies on clinical laboratory testing that delivers accurate and clinically relevant data (Vap et al., 2012; Arnold et al., 2019). Veterinary clinical laboratories play a critical role not only in diagnosing, classifying, and monitoring pathophysiological conditions, ruling out potential causes of illness, assessing disease progression or response to therapy, and detecting subclinical disorders in apparently healthy animals (Bush, 1991; Stockham & Scott, 2008), but also in supporting high-quality research. Their ability to generate reliable and standardized data makes them essential in research projects involving clinical patients, particularly in translational and comparative studies. In this context, veterinary laboratories serve as a vital link between clinical practice and scientific discovery, ensuring that research findings are grounded in accurate and reproducible laboratory results.
Unlike human clinical laboratories, veterinary laboratories are not subject to the same legislative and licensing requirements (e.g., ISO 15189), although the expectations for result quality are comparable. In fact, achieving high-quality results in veterinary settings can be more challenging due to interspecies variability (Lumsden, 2006; Vap et al., 2012). To address these challenges, the American Society for Veterinary Clinical Pathology (ASVCP) published the Quality Assurance Guidelines in 2019, which promote continuous quality improvement by identifying sources of laboratory error and offering practical tools for implementing comprehensive quality management systems (Arnold et al., 2019).

Laboratory testing is divided into three phases: preanalytical, analytical, and postanalytical. Errors can occur at any stage, but advances in instrumentation, automation, and quality control have significantly reduced analytical errors. Today, preanalytical errors are recognized as the leading cause of unreliable laboratory results in both human and veterinary medicine (Lippi et al., 2006; Hooijberg et al., 2012; Braun et al., 2015; Whipple et al., 2020). The ISO 15189 standard defines the preanalytical phase as encompassing all processes from the clinician's test request through sample collection, identification, and transport, up to the start of the analytical examination (Braun et al., 2015). Studies show that preanalytical errors account for the majority of laboratory mistakes, up to 75.3% according to Whipple et al. (2020), with most occurring outside the laboratory itself. Common issues include incorrect sample-to-anticoagulant ratios, clotted samples, in vitro hemolysis, mislabeling, and unlabeled specimens (Narayanan, 2003; Lippi et al., 2006; Zandecki et al., 2007a, 2007b; Lippi et al., 2008; Lippi et al., 2012; Upreti et al., 2013; Arnold et al., 2019; De la Salle, 2019; Whipple et al., 2020).

Advanced testing technologies cannot compensate for poor-quality samples. Therefore, proper collection, handling, and processing of blood samples are crucial to ensure the accuracy of laboratory results, prevent misinterpretation, and maintain high-quality outcomes in both patient care and research. This includes attention to sample integrity, appropriate tube selection, timely processing, and documentation of any preanalytical variables such as hemolysis, lipemia, or clotting. Adherence to standardized protocols minimizes variability and enhances reproducibility of results (Bush, 1991; NCCLS, 2003; Lippi et al., 2006; Hooijberg et al., 2012; Flatland & Vap, 2012; Vap et al., 2012; Arnold et al., 2019; Whipple et al., 2020).

3. Purpose, Scope and Applicability

The purpose of this SOP is to describe the procedures for the collection, handling, and processing of canine venous blood samples, as well as the performance of routine hematological and biochemical analyses. These procedures are part of the comprehensive evaluation of animals included in the Nanostructurome project.

The scope of this SOP includes step-by-step instructions for the collection of blood samples, preparation of serum and plasma samples, as well as the preparation of diluted ethylenediaminetetraacetic acid (EDTA) whole blood and EDTA plasma samples for the measurement and characterization of EVs. It also covers the determination of complete blood count (CBC) and white blood cell differential (DIFF), including parameters such as red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), hemoglobin concentration (HGB), hematocrit (HCT), and other related indices. In addition, the SOP outlines routine biochemical analyses for serum analytes including glucose, urea, creatinine, total protein, albumin, sodium (Na), potassium (K), chloride (Cl), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

This SOP is applicable to a wide range of basic and clinical research settings where blood-based analyses are performed. Its implementation is essential for minimizing preanalytical and analytical errors, thereby ensuring the generation of accurate, reliable, and reproducible laboratory results.

4. Health and safety warning

All personnel must adhere to established health and safety protocols when handling biological samples and operating laboratory equipment. Appropriate personal protective equipment (PPE), including laboratory coats, safety shoes, gloves, and, where necessary, masks and safety glasses, must be worn at all times. Since it is often impossible to determine whether a sample is infectious, all blood samples must be treated as potentially infectious and handled according to standard biosafety precautions.

Laboratory conditions must support safe and efficient work, including adequate lighting, ventilation, and ergonomic accommodations to minimize the risk of repetitive strain, prolonged sitting or standing, and other occupational injuries. Personal protective equipment must be appropriate for the specific procedures and equipment used in each laboratory area. Furthermore, veterinarians and veterinary technicians involved in the collection of

blood samples must adhere to established safety protocols and utilize PPE suitable for field conditions, ensuring the safety and integrity of the sampling process.
Notices, specialized labeling, and safety procedures for the handling, storage, and disposal of all samples, waste, and other supplies must be appropriate for the type of material. Personnel must receive comprehensive safety and biohazard training, including protocols for exposure to hazardous chemicals or infectious agents, prevention of bacterial contamination, awareness of zoonotic diseases, and emergency response procedures (e.g., fire or contamination events). All training must be documented, and staff must be aware of their responsibilities and the location of safety equipment (Laposata & Dighe, 2007; Arnold et al., 2019). In addition, all personnel are required to undergo a mandatory occupational safety examination every two years, in accordance with institutional and regulatory guidelines.

5. Cautions

Laboratory personnel must be informed of the hazards associated with individual reagents (as described in the original manufacturer instructions) and the potential risk of infection prior to beginning any work. The use of PPE is mandatory in the laboratory. Consumption of food and beverages is strictly prohibited. Direct contact of reagents with skin and eyes must be avoided, and reagents must never be ingested. All materials used must be disposed of in accordance with applicable regulations and established good laboratory and research practices. Written operating instructions for all equipment are available to users on site and must be followed at all times.
Veterinarians and veterinary technicians involved in the collection of blood samples must also be aware of biosafety risks and follow appropriate safety protocols. Personal protective equipment suitable for fieldwork, such as gloves, protective clothing, safety shoes, and sharps containers, must be used consistently to prevent exposure to infectious agents and ensure the safe handling and transport of biological samples.

6. Personnel Qualifications / Responsibilities

Personnel involved in this SOP include the following:
Experienced veterinarians or veterinary technicians: Responsible for the collection of blood samples, strictly adhering to established veterinary medical principles and guidelines.
Veterinarians or veterinary technicians: Responsible for the safe and timely transport of blood samples to the laboratory, ensuring sample integrity is maintained during transit.
Laboratory Personnel (including chemistry and veterinary technicians, and chemical engineers): Responsible for the handling and processing of blood samples, as well as the performance of routine hematological and biochemical analyses. All personnel must have completed appropriate training in analytical procedures, instrument operation and calibration, and data documentation.
Quality Control (QC) Officer (ISO/IEC 17025 accredited), also serving as the Head of Laboratory: Responsible for ensuring compliance with quality standards, reviewing analytical results, validating calibration and method performance (including control sample analysis), and identifying any deviations requiring corrective actions.
Laboratory Supervisor (Head of Laboratory): Responsible for overall adherence to procedures, including laboratory safety, regulatory compliance, equipment maintenance, and troubleshooting.
All personnel involved in the handling and processing of canine blood samples, as well as in the performance of hematological and biochemical analyses, must be adequately trained and regularly updated on laboratory procedures, with particular emphasis on minimizing preanalytical and analytical errors.
Laboratory staff are responsible for maintaining a clean and organized work environment, including proper care and routine cleaning of equipment and work surfaces. Any deviations from standard procedures must be clearly documented, preferably in both written and electronic formats (electronic information system - EasyVet), to ensure traceability and support quality assurance.

In addition, personnel must document any factors that could influence the interpretation of analytical results in **Appendix 1** (Record sheet for blood sample collection, handling, and preparation of diluted blood samples for EVs measurement and characterization) of this SOP. These include, but are not limited to, visible lipemia, hemolysis, icterus, the presence of blood clots, or insufficient sample volume. Accurate recording of such observations is essential for proper data interpretation and for maintaining the integrity of the analytical process.

All data must be securely stored and backed up in accordance with institutional data management policies (ISO/IEC 17025). Equipment must be operated and maintained according to the manufacturer's instructions, internal laboratory protocols and according to internal ISO/IEC 17025-based guidance document, V 77 – Equipment management. Regular maintenance and calibration are essential to ensure consistent performance and analytical reliability.

7. Materials, Equipment and Supplies

7.1. Materials and equipment required for venous blood samples collection, handling and processing, including the dilution of EDTA whole blood and EDTA plasma samples for the measurement and characterisation of EVs

2-mL tubes containing anticoagulant EDTA; 0.5-mL EDTA-containing microtainer tubes; 4-mL serum separator tubes; sterile needles or intravenous catheters, used in combination with syringe; appropriate PPE, such as lab coats, gloves, and safety shoes; an alcohol-based antiseptic; sterile gauze pads; adhesive tape or bandages to prevent bleeding; disposable protective gloves; a sharps disposal container; proper labeling materials, such as waterproof markers and pre-printed labels; Eppendorf tubes; pipette tips; waste disposal bins; plastic tubes; plastic cryovials for freezing serum and plasma samples at –80°C for long-term storage; dilution medium (ultraclean water, marine water, physiological saline (0.9% sodium chloride (NaCl) solution), or phosphate-buffered saline, depending on the sample); single-channel calibrated pipettes; and a centrifuge (Heraeus Megafuge 8R, Thermo Fisher Scientific).

7.2. Materials and equipment required for hematological analyses

the ADVIA 120 (SIEMENS) automated laser based hematology analyzer equipped with species-specific software; original, manufacturer-supplied reagents specific to the analyzer, including hemoglobin, basophil (BASO), RBC/PLT, and PEROX reagents, as well as diluents, lysing agents, and cleaning solutions; quality control whole blood materials at low, normal, and high levels; EDTA-anticoagulated whole blood samples; and appropriate PPE (lab coats, gloves, safety shoes).

7.3. Materials and equipment needed for biochemical analyses, including electrolytes

the RX Daytona (RANDOX) automated biochemical analyzer; RANDOX-specific, pre-prepared reagents for the determination of glucose, total protein, albumin, urea, and creatinine concentrations, as well as the enzymatic activities of ALP and ALT; RANDOX calibrators and quality control materials; RANDOX cleaning solutions and acid wash solution, distilled water and physiological saline solution for routine maintenance and prevention of contamination; a Roche electrolyte analyzer (9180, ROCHE) for the measurement of Na, K, and Cl concentrations; ROCHE-specific reagent and waste container (SnapPak cartridge) for electrolyte analyzer; other analyzer-specific solutions and control material: cleaning solution, sodium conditioning solution and ISETROL quality control solutions (three levels); dedicated plastic sample tubes used for transferring serum from the original collection tubes; and appropriate PPE (lab coats, gloves, safety shoes).

7.4. Extra equipment and consumables:

reagent reservoirs, waste disposal bins, 80°C freezer and refrigerator for sample and material storage, plastic tubes, distilled water/physiological saline (0.9 % NaCl) to dilute reagents and samples, especially when the measured values (concentrations, activities) are above the linear range of the assay.

8. Computer Hardware and Software

Hardware: desktop computers; software: **Hematological analysis**: Multispecies system software 6.9.0-MS (ADVIA 120 Hematology System; Siemens Healthcare Diagnostics, Munich, Germany); **Biochemical Analysis**: RX Daytona (RANDOX) analyzer-specific software with a built-in user interface for operation and data management.

9. Step by Step Procedures

9.1. Blood samples collection, handling and processing

9.1.1. Blood samples collection

- Blood samples must be collected prior to any therapeutic or diagnostic procedures (e.g., surgery, infusion, transfusion, biopsy, endoscopy).
- Collect venous blood from the jugular vein using sterile needles or intravenous catheters with syringes.
- Ensure animals are fasted before collection for at least 12 hours.
- Shave the area around the intended puncture site to ensure clear access and reduce contamination risk.
- Disinfect the puncture site with an alcohol-based antiseptic.
- After blood collection, apply sterile gauze to the puncture site and secure with adhesive tape or a bandage.
- Wear disposable gloves throughout the procedure and dispose of used needles in an approved sharps container.
- Use waterproof markers or pre-printed labels for accurate sample identification.
- Confirm that the sample is collected from the correct animal, as indicated on the request form.
- When collecting multiple tubes during a single venipuncture, follow the recommended order of draw:
  - Blood culture tube
  - Coagulation tube (blue stopper)
  - Serum tube (red or yellow-red stopper)
  - Heparin tube (green stopper)
  - EDTA tube (lavender stopper)
  - Glycolytic inhibitor tube (grey stopper)

9.1.2. Sample transport and initial handling

- Transport samples to the in-house laboratory at room temperature as quickly as possible.
- Upon arrival:
  - EDTA whole blood samples (0.5-mL tubs for hematology): place them in a blood mixer for at least 30 minutes before analysis. Analysis should be performed within 1 hour of collection.
  - EDTA whole blood samples for obtaining EDTA plasma (2-mL tubes for preparation of diluted samples for EVs analysis): prepare diluted EDTA whole blood samples and then centrifuge immediately at 1500 × g for 15 minutes at room temperature. Transfer plasma to Eppendorf tubes or cryovials for further processing and storage, respectively.
  - Serum separator tubes for obtaining serum (4-mL serum separator tubes for biochemistry): allow to clot at room temperature (22–25°C) for 20–30 minutes. Keep tubes upright to promote clotting and minimize hemolysis. After clotting, centrifuge serum tubes at 1300 × g for 10 minutes at room temperature. Perform biochemical analyses within 2 hours of blood collection.

9.1.3. Preparation of diluted blood samples for EVs measurement and characterization

- Dilution of EDTA whole blood:
  - Gently invert the EDTA blood collection tube 8 times to ensure homogeneity before pipetting. Then, prepare 1:10 dilution by mixing 100 µL of EDTA whole blood with 900 µL of physiological saline solution.

- Dilution of EDTA plasma:
  - Mix 4 µL of EDTA plasma with 196 µL of physiological saline solution (1:50 dilution).
- Keep prepared diluted blood samples at room temperature until transported for EVs analysis. To preserve the integrity of extracellular vesicles, the diluted samples must remain undisturbed; avoid vigorous shaking or agitation. Samples should be kept in a stable, upright position to minimize mechanical stress and prevent vesicle disruption.

9.2. Hematological analyses using automated hematological analyzer ADVIA 120

9.2.1. Preparation

- Before starting the analyzer, check that all required reagents are available in sufficient quantities and are within their expiration dates.
- Ensure that the waste container is empty or has enough capacity for the upcoming analysis.
- Power on the ADVIA 120 analyzer and allow it to complete its initialization and self-check, then run the quality control (QC) samples.
- Document all required information in the Appendix 11 ('Record of hematological analyses') of the Quality Manual (QM 15) of the Small Animal Clinic. This includes consecutive analysis number, date of analysis, owner's name, species, animal's name, animal's file number and type of hematological analysis required.

9.2.2. Quality Control

- Run QC samples using manufacturer-provided blood controls (low, normal, and high; all from SIEMENS) in accordance with the manufacturer instructions.
- Review QC results to confirm they fall within acceptable ranges.
- If QC fails, troubleshoot according to the analyzer's user manual before proceeding with patient samples.

9.2.3. Sample Analysis

- Prior to analysis, the sample must be placed on a blood mixer for approximately 15–20 minutes to ensure proper homogenization.
- Immediately before aspiration, gently invert the tube 8 times.
- Initiate the analysis through the veterinary software interface connected to the ADVIA 120 hematology analyzer. Select the appropriate test parameters (CBC and DIFF) and ensure the correct animal species (canine mode) is selected.
- Run the EDTA whole blood sample using the manual aspiration port. The analyzer will automatically perform the CBC and DIFF analysis.
- Wait for the analysis to complete and review the results on display.

9.2.4. Result Verification and Documentation

- Verify that all parameters are within expected physiological ranges.
- Flag any abnormal results for repeat analysis or further investigation.
- Save and export results to the electronic information system (EasyVet) and print them for manual documentation.
- Store the analyzed EDTA whole blood samples for 24 hours at 2–8°C to allow for possible re-analysis if needed.

9.2.5. Post-Analysis

- At the end of each analysis session, perform routine maintenance as prompted by the analyzer.
- Routine daily cleaning cycles are automated and pre-programmed within the ADVIA 120 system.
- Perform weekly and monthly manual cleaning procedures according to the manufacturer's maintenance schedule and guidelines.
- Maintenance activities are not recorded in a physical instrument logbook; instead, the analyzer's internal system tracks routine operations. In addition, weekly maintenance of the ADVIA 120 hematology analyzer is documented in Appendix 13 ('Weekly maintenance of the hematological analyzer ADVIA 120')

of the QM 15, while monthly maintenance is documented in Appendix 14 ('Monthly maintenance of the hematological analyzer ADVIA 120') of the QM 15.

- At the end of the day, initiate the "End of Day" procedure within the ADVIA 120 software to store all results related to routine samples and control sample analyses.

9.3. Biochemical analyses, excluding electrolyte, using automated biochemical analyzer RX-Daytona

9.3.1. Preparation

- The biochemical analyzer is programmed to automatically exit sleep mode and begin its initialization process at 5:30 a.m. each day. Allow the system to complete its full startup sequence, including any scheduled pre-analytical maintenance procedures. These typically include: cuvette wash (2 cycles) and priming (2 cycles).
- Verify that all reagents, calibrators, and QC materials are available and within their expiration dates.
- Load the reagents onto the reagent carousel and scan their barcodes to ensure proper identification and traceability.
- Gently mix serum or plasma samples to ensure homogeneity. Visually inspect and document any pre-analytical factors such as lipemia, hemolysis, or icterus.

9.3.2. Quality Control

- Run QC samples (low or normal or high) according to manufacturer instructions.
- If QC results fall outside acceptable ranges:
  - Re-run QC to confirm the deviation.
  - If still out of range, run Randox calibrators to re-establish the calibration curve.
  - We can proceed with patient samples when QC results are within acceptable limits.

9.3.3. Sample Analysis

- Use the RX Daytona software interface to select the appropriate biochemical tests (e.g., glucose, urea, creatinine, total protein, albumin, ALT, ALP) based on the sample type and the veterinarian's diagnostic request.
- Assign the selected tests to the corresponding sample positions on the sample carousel.
- Load the prepared serum (plasma or urine samples) into the designated positions on the sample carousel.
- Initiate the analysis. The RX Daytona will automatically perform the selected biochemical tests, including reagent handling, sample aspiration, mixing, incubation, and photometric reading.
- Store remaining samples according to the type of analysis:
- Routine diagnostic samples are stored in a refrigerator (2–8°C) for up to 2 weeks.
- Research samples are stored in a -80°C deep freezer, in accordance with the specific research protocol requirements.

9.3.4. Result Handling

- Review all analytical results for accuracy, ensuring they fall within expected physiological or reference ranges. Flag any abnormal or unexpected values for repeat analysis or further investigation.
- Validated results are exported to electronic information system EasyVet. A hard copy is not printed; instead, results are manually transcribed onto:
  - the 'Blood sample request form' (Appendix 21 of the QM 15), and
  - the 'Documentation of the Results of Biochemical Tests' form, which is Appendix 16 of QM 15.

9.3.5. Post-Analysis

- Perform routine maintenance and cleaning procedures as prompted by the analyzer, including tasks such as probe washing, waste disposal, and reagent cooling checks.
- At the end of the working day, store all reagents in the refrigerator according to manufacturer guidelines to maintain stability and performance.
- Document all maintenance and calibration activities electronically within the analyzer's internal system or designated digital records, as a physical instrument logbook is not used.
- Ensure that the analyzer is placed in sleep mode at the end of the working day. This process includes several end-of-day procedures such as system flushing, probe cleaning, and temperature stabilization, as guided by the analyzer's software prompts.

9.4. Electrolyte analyses using 9180 ROCHE electrolyte analyzer

9.4.1. Preparation

- Exit Standby mode and ensure the Roche 9180 electrolyte analyzer has completed initialization
- Verify that the SnapPak, cleaning solution, sodium conditioning solution, and ISETROL three-level control materials are available and within expiration dates.
- Perform cleaning, sodium electrode conditioning and run calibration. Ensure the analyzer has completed its daily self-check and is ready for operation.
- Gently mix serum or plasma samples to ensure homogeneity. Visually inspect for hemolysis, lipemia, or icterus and document any abnormalities.

9.4.2. Quality Control

- Immediately after calibration, run quality control samples (low or normal or high) using ROCHE ISETROL controls, following the manufacturer's instructions.
- If QC results fall outside acceptable limits:
  - Repeat the QC test to confirm the deviation.
  - If results remain out of range, recalibrate the analyzer.
  - If QC results are still outside acceptable limits after recalibration, contact Roche service for further assistance.
  - Proceed with patient sample analysis only when QC results are within acceptable limits.

9.4.3. Sample Analysis

- Use the analyzer's touch-screen interface to select the appropriate sample type and analysis (Na, K, Cl).
- Aspirate the serum (or plasma or urine) sample manually using the sample probe. Wipe the probe immediately after sample aspiration.
- The analyzer will automatically perform the measurement and display the results.
- Repeat the analysis if prompted by the system due to sample or measurement error.

9.4.4. Result Handling

- Review the results on the analyzer display and verify they fall within expected physiological or reference ranges. Flag any abnormal or unexpected values for repeat analysis or further investigation.
- Transcribe validated results manually onto:
  - the 'Blood sample request form' (Appendix 21 of QM 15), and
  - the 'Documentation of the results of biochemical tests' form, which is Appendix 16 of QM 15.

- Results are printed but not exported electronically to EasyVet, so enter the results manually into the EasyVet system.

9.4.5. Post-Analysis

- The analyzer performs automatic cleaning after each analysis according to preprogrammed cleaning cycles.
- Manual cleaning is not required, except for wiping the probe immediately after aspiration, which is already included in the analysis procedure.
- Calibration is preprogrammed to occur three times per day, so no manual calibration is needed at the end of the working day.
- At the end of the working day, place the analyzer in standby mode.

9.5. Data acquisition

9.5.1. Hematological analysis using automated hematological analyzer ADVIA 120

The ADVIA 120 hematology analyzer performs CBC, DIFF, and reticulocyte measurements using flow cytometry, laser-based optical detection, and cytochemical staining. Results are generated electronically and include numerical values, histograms, and cytograms (scatter plots), which provide visual representations of cell populations and distributions. Numerical values are automatically transferred to the electronic information system EasyVet for archiving, clinical evaluation, and research purposes. In addition, results are printed for documentation and a backup of all outputs is stored on the hard disk of the ADVIA 120, ensuring data integrity, traceability, and compliance with regulatory requirements.

Red blood cell counts and PLT are analyzed using multi-angle light scatter technology to assess cell size and internal complexity. Hemoglobin concentration is determined photometrically following the lysis of red blood cells. Additional parameters, including HCT, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), are calculated based on direct measurements.

White blood cells are stained with cytochemical reagents that highlight specific intracellular components (e.g., peroxidase). The stained cells are passed through a flow cell and analyzed using laser-based optical detection. The system measures:

- axial light loss (cell size),
- side scatter (internal complexity),
- absorbance (cytochemical staining intensity).

The ADVIA 120 is connected to the electronic information system EasyVet, to which it transmits numerical results electronically via a standardized communication protocol. This process constitutes electronic archiving of hematological data, ensuring secure storage and traceability in compliance with quality and regulatory standards.

9.5.2. Biochemical analysis using automated biochemical analyzer RX-Daytona and elec

trolyte analyzer 9180 ROCHE

The RX-Daytona performs biochemical analyses using photometric and turbidimetric methods. Results are generated digitally and include numerical values and reaction curves. Quantification of analytes is based on calibration curves, which are established by measuring the absorbance of known calibrator concentrations. The analyzer uses these calibration curves to determine the unknown concentration or activity of analytes in patient samples by comparing the measured absorbance of the test sample to the absorbance values of known calibrators plotted on the standard curve.

Concentrations of electrolytes (Na, K, Cl) are measured using the 9180 Roche electrolyte analyzer, which operates on the principle of direct potentiometry with ion-selective electrodes (ISE). The results are printed and manually transcribed into the EasyVet electronic information system and onto the Blood Sample Request Form (Appendix 21 to QM 15) for documentation and clinical interpretation.

The results of biochemical analyses (numerical values of concentrations and activities) are generated in digital format and are electronically transmitted to the EasyVet information system, where they are archived for clinical and research use.

A backup of the results is also retained on the RX-Daytona biochemical analyzer. In the case of analyses performed for patients included in research studies, results are additionally manually transcribed into the following documentation:

- The Blood Sample Request Form (Appendix 21 of QM 15), for internal tracking and clinical correlation.
- The Documentation of the Results of Biochemical Tests form (Appendix 16 of QM 15), for inclusion in patient records and to ensure audit readiness.

The results of biochemical analyses (numerical values) are available in three formats: electronically via EasyVet and as a backup on the biochemical analyzer, on the 'Blood sample request form' (Appendix 21 of QM 15), and on the 'Documentation of the results of biochemical tests form' (Appendix 16 of QM 15).

9.6. Troubleshooting

9.6.1. Collection, handling and processing of blood samples

Collection of blood into 0.5-mL EDTA tubes:

- Insufficient volume: may lead to clotting or inaccurate cell counts.
- Clot formation: due to delayed mixing or underfilling.
- Hemolysis: caused by rough handling or small needle gauge.

Troubleshooting recommendations:

- Ensure proper vein selection and use of appropriate needle size (ideally 21–23G).
- Gently invert the tube 8–10 times immediately after collection.
- If volume is low, prioritize this tube for CBC as it requires minimal blood.

Collection of blood into 2-mL EDTA tubes:

- Underfilling: affects plasma yield and EVs analysis.
- Delayed processing: can alter EVs profile and plasma quality.
- Clotting: due to improper mixing.

Troubleshooting recommendations:

- Collect this tube second to avoid platelet activation from earlier draws.
- Mix gently and process within the recommended time frame (usually <1 hour).
- If volume is insufficient, prioritize EVs or plasma based on study protocol.

Collection of blood into 4-mL serum separator tubes:

- Non-compliance with fasting: affects lipemia and analyte levels.
- Hemolysis, lipemia, icterus: interfere with biochemical assays.
- Insufficient clotting time: leads to fibrin strands in serum.
- Low volume: inadequate for both routine and storage needs.

Troubleshooting recommendations:

- Ensure 12 hour fasting prior to collection.
- Allow full clotting (20–30 min) before centrifugation.
- Use clean venipuncture techniques to avoid hemolysis.
- If volume is low, prioritize routine biochemistry and freeze fewer aliquots.

The checklists below provide troubleshooting guidance for the preparation of diluted EDTA whole blood (Table 1) and plasma samples (Table 2), as well as sample handling and transportation (Table 3) of samples for the EVs analysis. Checklists address common issues, their possible causes, and recommended solutions.

Table 1. Dilution of EDTA Whole Blood (1:10)

Issue	Possible Cause	Troubleshooting
Inhomogeneous sample	Inadequate mixing of EDTA tube	Gently invert the blood tube exactly 8 times before pipetting. Avoid vortexing.
Incorrect dilution ratio	Pipetting error	Use calibrated pipettes and verify volumes. Perform dilution in a clean, labeled microtube.
Clotting in sample	Delayed mixing after collection or underfilling of EDTA tube	Ensure immediate and proper mixing post-collection. Discard clotted samples.
Hemolysis	Rough pipetting or small needle during collection	Use gentle pipetting and proper venipuncture technique. Discard visibly hemolyzed samples.

Table 2. Dilution of EDTA Plasma (1:50)

Issue	Possible Cause	Troubleshooting
Pipetting inaccuracy	Very small volume (4 µL)	Use precision pipettes (e.g., P10) and low-retention tips. Pre-wet tips to improve accuracy.
Contamination	Unclean pipette tips or tubes	Use sterile, nuclease-free consumables. Work in a clean environment.
Plasma degradation	Delay in processing or improper storage	Process plasma promptly and freeze the remained plasma as soon as possible at – 80°C.

Table 3. Sample Handling and Transport for EVs Analysis

Issue	Possible Cause	Troubleshooting
Vesicle disruption	Shaking, agitation, or vortexing	Handle tubes gently. Use racks to keep samples upright and stable.
Temperature fluctuations	Exposure to heat or cold	Maintain room temperature (18–25°C). Avoid placing near heat sources or in direct sunlight.
Sample mix-up	Inadequate labeling	Label tubes immediately after preparation with sample ID, dilution, and time. Double-check before transport.

9.6.2. Hematological analyses

Detailed troubleshooting procedures for hematological analyses using the ADVIA 120 analyzer are available directly within the analyzer's software interface and in the printed user manual provided by the manufacturer; due to their complexity and length, they are not included in this SOP.

9.6.3. Biochemical analyses

Detailed troubleshooting procedures for biochemical and electrolyte analyses using the RX-Daytona and 9180 Roche analyzers, respectively, are available within the respective analyzer software interfaces (RX Daytona) and in the printed user manuals provided by the manufacturers (RANDOX, ROCHE); due to their complexity, they are not included in this SOP.

10. Data and records management

Data and records management related to blood sample collection, handling, and the preparation of diluted blood samples for EVs measurement and characterization are detailed in Appendix 1 ('Record sheet for blood sample collection, handling, and preparation of diluted blood samples for EVs measurement and characterization') of this SOP.

Upon completion of hematological analysis, the results are immediately and automatically saved in multiple formats to ensure accessibility, traceability, and compliance with documentation standards (ISO/IEC 17025).

- Numerical values are electronically transmitted to the EasyVet information system, where they are archived for clinical interpretation and long-term storage.
- Numerical values, histograms, and cytograms are simultaneously stored on the hard disk of the hematology analyzer as a local backup.
- Hard copies of the results are always printed for archiving and documentation purposes.

In the case of research, the printed hard copy of the results is stapled to the 'Blood sample request form' (Appendix 21 of QM 15), which contains all necessary information regarding the patient and the patient's owner. This form also serves as a record for documenting any deviations related to blood sample integrity, such as the presence of clots, insufficient volume, or other anomalies. This ensures complete traceability and proper linkage of analytical results with subject-specific data.
All forms of saved results—digital and printed—are used for:

- Archiving and documentation (e.g., for audits, regulatory compliance, or data backup),
- Internal tracking and clinical correlation, and
- Availability to clinicians for timely interpretation and decision-making.

Upon completion of biochemical analysis, results are automatically saved in electronic form in two locations:

- On the RX-Daytona biochemical analyzer, where data includes:
  - Concentrations and activities of analytes,
  - Quality control measurements, and
  - Calibration curve data.
- In the EasyVet electronic information system, where only the numerical results of concentrations and activities are archived. These results are not only stored for long-term record-keeping but are also immediately available to clinicians for interpretation and clinical decision-making.

Although results are electronically archived, all test results are also manually transcribed into the following documentation forms:

- The 'Documentation of the results of biochemical tests form' (Appendix 16 of QM 15), for inclusion in patient records and to ensure audit readiness.
- The 'Blood sample request form' (Appendix 21 of QM 15), for internal tracking and clinical correlation in the case of analyses performed for patients included in research studies. This form also serves to document any deviations related to serum sample integrity, such as hemolysis, lipemia, icterus, or insufficient sample volume, thereby supporting traceability and quality assurance.

This multi-format, dual-location data management system ensures that hematological and biochemical results are securely stored, traceable, and readily accessible—both digitally and in print—for clinical interpretation, regulatory compliance, internal tracking, and research documentation
In accordance with ISO/IEC 17025 accreditation requirements, all hematological and biochemical results, whether stored digitally in the EasyVet system and on analyzers or retained as printed hard copies, are archived for a minimum of six (6) years, ensuring long-term traceability, data integrity, and full compliance with applicable regulatory standards.

11. Waste management

The waste includes disposed gloves, Eppendorf tubes where the samples are diluted, plastic pipette tips, used paper napkins for cleaning the observation chamber and potential sample spill and remnants of the samples. Disposal material should be divided into ap-

propriate waste fractions, according to applicable laws and good research laboratory practice. Potentially hazardous materials should be placed in special containers and delivered to the relevant acquisition units.

All waste generated during blood collection, handling, and processing—as well as from hematological and biochemical analyses—is managed in strict accordance with the veterinary waste classification scheme and applicable national and EU regulations.

- Infectious and potentially infectious waste is classified under EWC (European Waste Code) code 18 02 02* and includes materials such as used blood collection tubes, contaminated gloves, and other disposables that may pose a biological hazard.
- Sharp materials (e.g., needles, lancets, scalpels, and other sharp instruments not contaminated with hazardous substances) are classified under EWC code 18 02 01 and are disposed of in puncture-resistant sharps containers.
- Liquid chemical waste, including used reagents and cleaning solutions from biochemical and hematological analyzers, is classified under EWC code 18 02 05.

All waste is carefully sorted at the point of generation and stored in designated, clearly labeled containers. Waste disposal is handled by a certified external waste management company. Full documentation is maintained for each waste collection, including:

- Tracking numbers
- Mass of each waste category
- Collection dates
- Certificates of disposal

This documentation ensures full traceability and compliance with environmental and biosafety regulations.

12. Related protocols or SOPS

This SOP integrates a combination of optimized, well-established procedures for the collection, handling, and processing of canine venous blood samples, as well as the performance of hematological and biochemical analyses. The optimization of these procedures enhances sample quality, improves analytical reliability, and supports consistent laboratory performance. In addition to these procedures, this SOP aligns with several internal ISO/IEC 17025-based guidance documents developed at the Veterinary Faculty, University of Ljubljana, as part of the faculty's accreditation process. These documents are an integral part of the faculty's quality management system and are strictly followed in daily laboratory practice, including research activities involving numerous patients enrolled in various scientific studies.

The following internal guidance documents are referenced in the implementation of this SOP. They form an integral part of the Veterinary Faculty's quality management system and are routinely followed in daily laboratory operations, including research activities involving patient participation:

- V 67 – Document and data control related to the quality management system
- V 77 – Equipment management
- V 79 – Selection, onboarding, and training of personnel
- V 82 – Handling of temperature-controlled equipment
- V 83 – Preparation of test reports
- V 86 – Procurement procedures
- V 87 – Handling complaints and monitoring client feedback
- V 96 – Pipette verification
- V 102 – Preparation of control charts
- V 104 – Thermometer calibration
- V 110 – Validation and verification of chemical analytical methods
- V 162 – Management of nonconformities, risks, opportunities, and corrective actions
- V 163 – Preparation and review of testing offers and contracts
- V 178 – Reception, identification, handling of patients, and traceability
- V 180 – Pipette calibration

Confidentiality Notice:
These internal documents are confidential and intended solely for use within the Veterinary Faculty. They must not be shared, distributed, or reproduced outside the institution without proper authorization.

13. Quality control and quality assurance section

13.1. Instrument calibration and quality control

All instruments (analyzers) used in this SOP are regularly maintained and calibrated by trained service technicians and laboratory personnel. The analyzers are serviced annually by qualified technicians from the supplier, and all calibrations are performed by trained laboratory staff to ensure accuracy and reliability.
Daily quality control is performed on all analyzers using analyzer-specific control materials prior to the analysis of patient samples (see quality control procedures described in the step-by-step section).

13.2. Critical processes parameters and checkpoints

A critical step in this SOP is the correct collection of blood samples, as previously described. The quality of analytical results is directly dependent on the quality of the collected samples; poor sample quality often leads to inaccurate results and misinterpretation. Accurate pipetting is essential for the preparation of diluted EDTA whole blood and plasma samples used for EVs measurement and characterization. Therefore, only precisely calibrated pipettes should be used.
Sample handling and storage conditions are also critical. Prepared samples must be handled gently, as shaking or agitation can disrupt the vesicles. Additionally, diluted samples should not be refrigerated, as low temperatures may compromise vesicle integrity.
Reagent storage and preparation are further critical factors. Although these fall within the analytical phase, they are controlled through regular calibration and the analysis of quality control samples, which help prevent significant analytical errors.

14. Data on procedures and samples

Data on procedures and samples are given in **Table 4**.

Table 4. Data on procedures and samples

Description of the outcome	Prepared diluted EDTA whole blood and EDTA plasma samples. Results include hematological analysis (CBC + DIFF) and biochemical analysis (Na, K, Cl, urea, creatinine, glucose, ALT, ALP, total protein, and albumin).
Time required to obtain the results	15–20 minutes per sample for collection, depending on patient compliance; 10–15 minutes for preparation of diluted blood samples (excluding centrifugation); 5–10 minutes for hematological analysis; and 10–15 minutes for biochemical analysis.
Volume of the sample needed	At least 100 µL of EDTA whole blood and 4 µL of EDTA plasma are required for the preparation of diluted samples (EVs); at least 0.5 mL of EDTA whole blood is needed for hematological analysis; at least 300 µL of serum is required for biochemical analysis, including electrolytes (Na, K, Cl); and at least 300 µL of EDTA plasma and 3 × 300 µL of serum samples are needed for further analyses (to be stored at –80 °C).
Estimated cost without manpower	60 EUR/sample, primarily covering the cost of reagents, analyzer maintenance, and consumables.
Contact person	Alenka Nemec Svete, alenka.nemecsvete@vf.uni-lj.si

Conclusions

This SOP provides a comprehensive framework for the standardized collection, handling, and processing of canine venous blood samples, ensuring the integrity and reliability of laboratory analyses. By addressing both preanalytical and analytical phases, the SOP minimizes variability and reduces the risk of errors that could compromise data quality.

The detailed protocols for preparing serum, plasma, and diluted EDTA whole blood and EDTA plasma samples, along with procedures for hematological and biochemical analyses, support the generation of accurate and reproducible results. These outcomes are essential for the clinical evaluation of animal patients and for advancing research objectives within the Nanostructurome project.

Through consistent implementation by trained personnel, this SOP enhances laboratory performance, supports high-quality veterinary care, and contributes to the success of translational and comparative research. It reinforces the critical role of veterinary laboratories in bridging clinical practice and scientific discovery.

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Appendix 1: Record sheet for blood sample collection, handling, and preparation of diluted blood samples for EVs measurement and characterization

Patient and Owner Information

- Attach patient and owner identification sticker.

I. Blood samples collection (Total volume: 6.5 mL)

- 1x 0.5-mL EDTA tube: CBC + DIFF
- 1x 2-mL EDTA tube:
  - EVs - preparation of diluted EDTA whole blood samples and preparation of diluted EDTA plasma samples
  - 1 x 300 µL of EDTA plasma for further analyses (stored at – 80°C)
- 1x 4-mL serum separator tube without inert gel:
  - 300 µL of serum for biochemical analysis (urea, creatinine, total proteins, albumins, ALT, ALP, Na, K, Cl)
  - 3 x 300 µL for further analyses (stored at – 80°C)

II. Sample handling, processing and preparation of diluted samples for EVs measurement and characterization

Please complete the table below to document sample status and any relevant observations.

Sample	Obtained (YES/NO)	Comments (HIL, small samples volume, other)
0.5-mL EDTA tube		
2-mL EDTA tube		
4-mL serum separator tube		
Diluted EDTA whole blood		
Diluted EDTA plasma		
EDTA plasma		
Serum		

HIL, hemolysis, icterus, lipemia