NATIONAL CENTRE FOR ANIMAL HEALTH

NATIONAL VETERINARY LABORATORY

STANDARD OPERATING PROCEDURE

SOP No: NCAH/LSU/BIOCHEM 01
Title: SOP on Serum Collection for Biochemistry Tests
Version No: 3, Total Pages:4
Issue Month/Effective Date: May 2026
Revision: Summary:
Supersedes Version No: 2
Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL, DVL

1. Scope

The purpose of this SOP is to describe the general method for collection of serum samples for the biochemical tests.

2. Objective

To guide the laboratory technicians in collecting and preserving appropriate samples for laboratory investigations for biochemistry tests

3. General information/Responsibility

Serum biochemical analysis consists of looking into the variation in the levels of

3.1. Serum total protein
3.2. Macro and micro minerals
3.3. Glucose
3.4. Triglycerides
3.5. Hormones
3.6. Cholesterol
3.7. Creatinine
3.8. Bilirubin
3.9. Ketone bodies
3.10. SGPT
3.11. SGOT
3.12. Urea
3.13. Alkaline phosphatase
3.14. Albumin
3.15. Uric acid

4. Equipment and Consumables

4.1. Blood vacuum tubes, 5-10ml: These should be silicone coated or Gel coated vacutainers, without anticoagulant (red capped).
4.2. Sterile 5-10ml syringe
4.3. Gloves
4.4. Spirit cotton swab
4.5. Scissor or clipper
4.6. Torniquet
4.7. Hypodermic needle (21-16G)
4.8. Adaptor

5. Procedure:

5.1. Collection of serum samples

5.1.1. Collect the blood in a tube from the living patients. It should not be agitated and kept in a cool place. Dry syringes are to be used for collection in order to avoid hemolysis. The amount of blood to be collected is approximately 5 ml in large animals, 3 ml in companion animals and 2 ml in case of poultry.

5.1.2. Keep the tube at an angle. Clotting of blood will take place in 1-2 hours and serum separation depends upon the temperature and it is separated in about 2-24 hours

and a clear serum appears in the tube. Sometimes I need to use a centrifuge to separate serum at 1500-2000rpm for 5mins.

5.1.3. Pipette out the serum in a serum vial. Stopper it and after labelling, dispatch to the lab with a forwarding letter or fill the sample or Laboratory request form

(While preparing serum, care should be taken to prevent hemolysis. Otherwise, the serum becomes useless and unfit for tests).

5.2. **Labelling samples**

5.2.1. Samples must be labelled serially (1- 30) with a waterproof pen, preferably on an adhesive label. Keep a key list, which correlates sample numbers with animal identification. Do not label the stopper, which is removed during testing.

5.2.2. The specimen advice submitted with the samples should list clinical details beside each sample number. This will allow the laboratory to offer an informed comment on the results and perhaps apply other relevant tests.

5.2.3. Do not label samples with tag numbers, names, etc., as this leads to confusion and errors in reading numbers in the laboratory. It also makes it very difficult for the laboratory to ensure all samples are present or to check on missing or broken samples.

5.2.4. DO NOT label containers with water- soluble ink. It smudges when wet and may rub off if samples are chilled or frozen.

5.2.5. Serum can be frozen, provided there are no blood cells present in it.

5.3. **Storage of specimens prior to dispatch**

5.3.1. Samples should be allowed to clot before transporting them over any distance.

5.3.2. Clots may not retract readily in cold weather or if they are chilled too soon after collection.

5.3.3. Sample should be held in a warm room until the clot is retracted.

5.3.4. Once the clot has retracted, blood samples must be held chilled to reduce contamination, hemolysis and autolysis.

5.3.5. If the laboratory will not get the samples within 48 hours of collection, decant the serum into a 5ml sterile, screw-capped plastic container and submit the serum sample only.

5.3.6. If virological examination of the clot is also needed (Pestivirus antigen detection), submit the clot separately.

6. Safety

Use a separate sterile needle to avoid mechanically transmitting infectious agents from one animal to another. The samples should be considered infectious since some of the bacterial pathogens could be zoonotic in nature.

7. Waste Disposal

All the waste generated during sample collection should be disposed appropriately based on the zoonotic and non-zoonotic nature of the cases.

8. References

- USAID PREDICT. Marcela Uhart, University of California, Davis (2016) Livestock Sampling Methods: Cattle, Sheep, Goats, Camels, and Swine
- Standard operating procedures. 2023. Queensland animal ethical committee. P 16.

SOP No: NCAH/LSU/BIOCHEM 02
Title: SOP on Urine Collection for Biochemistry Tests
Version No: 3, Total Pages: 4
Issue Month/Effective Date: May 2026
Revision Summary: Annexure
Supersedes Version No: 2
Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution:NCAH, NVH, RVH, SVL,DVL

1. **Scope**

The purpose of this SOP is to describe the general method for collection of urine samples for biochemical tests.

2. **Objective**

To guide the laboratory technicians in collecting and preserving appropriate samples for laboratory investigations for biochemistry tests

3. **General information/Responsibility**

Urine samples need to be collected to get valuable information in regard to function of the urinary system (kidney) as well as other organs/ systems of the body. Urine needs to be collected in case of Haematuria and should be differentiated from haemoglobinuria. Some of the conditions where urinalysis is carried out is as follows:

3.1.*Disease conditions*

- 3.1.1. Renal disease
- 3.1.2. Bladder infection
- 3.1.3. Lower urinary tract infection
- 3.1.4. Neoplasm
- 3.1.5. Liver diseases
- 3.1.6. Diabetes
- 3.1.7. Ketosis in ruminants
- 3.1.8. Acidosis and Alkalosis

3.2.*Leptospiral* infection

- 3.2.1. Hormonal assay
- 3.2.2. Kidney worms

3.3.*Urine analysis*

- 3.3.1. Ketone bodies
- 3.3.2. Glucose
- 3.3.3. Bilirubin
- 3.3.4. Protein
- 3.3.5. Bile pigments
- 3.3.6. Blood (WBC & RBC)
- 3.3.7. Casts
- 3.3.8. pH
- 3.3.9. Cells

4. **Equipment/materials**

4.1. Clean glass or sterile plastic vials with screw capped lids,
4.2. Urinary catheter,
4.3. Sterile syringe (5-10ml),
4.4. Gloves
4.5. Cotton swab
4.6.Shaving blade or hair clipper

5. **Reagents, solution and buffer**

5.1.Toluene- It can be added in sufficient quantities to form a film on the surface. It can

preserve urine for 24 hours. But it has the drawback that it brings changes to Ketone bodies.

5.2. Formalin- One or two drops of formalin for 30 ml of urine can be added but it may give false positives for the sugar.

5.3. Thymol- It can also be used at 0.1gm/100ml of urine. But it gives a false positive reaction to albumin.

5.4. Boric acid- It can also be used at the rate of 0.5g/28ml of urine. This will prevent bacterial multiplication for 4 days**.**

**For cytological studies, urine is collected by catheterization and 40% ethyl alcohol is used as a preservative. In suspected cases of leptospirosis, urine should be made alkaline and then dispatched.*

6. Procedure

Manual stimulation - ensure sufficient restraint to prevent the animals (animals) from moving away during the procedure. Gently massage the skin under the vulva (escutcheon). In most cases this will result in urine flow in less than one minute.

6.1. It is best to collect the urine in the early morning, however if this is not possible, it should be collected 3 hours after the meal.

6.2. Always collect midstream urine or discard the first stream of urine.

6.3. If possible, collect it directly from the urinary bladder by catheterization or cystocentesis (refer annexure 1).

6.4. Urine can be collected in a clean container like glass vials or disposable plastic container; opaque plastic or dark glass should be used if the specimen is not examined soon after collection.

6.5. Urine samples can be preserved under refrigeration for 2-3 hours. The preservatives can be added to the urine sample if immediate analysis or refrigeration is not possible.

6.6. Dispatch to the lab with a forwarding letter or fill the Laboratory request form (refer annexure 2).

7. Safety

Urine may contain infectious pathogens that may infect humans hence, it should be handled with care.

Troubleshoot

1. NA

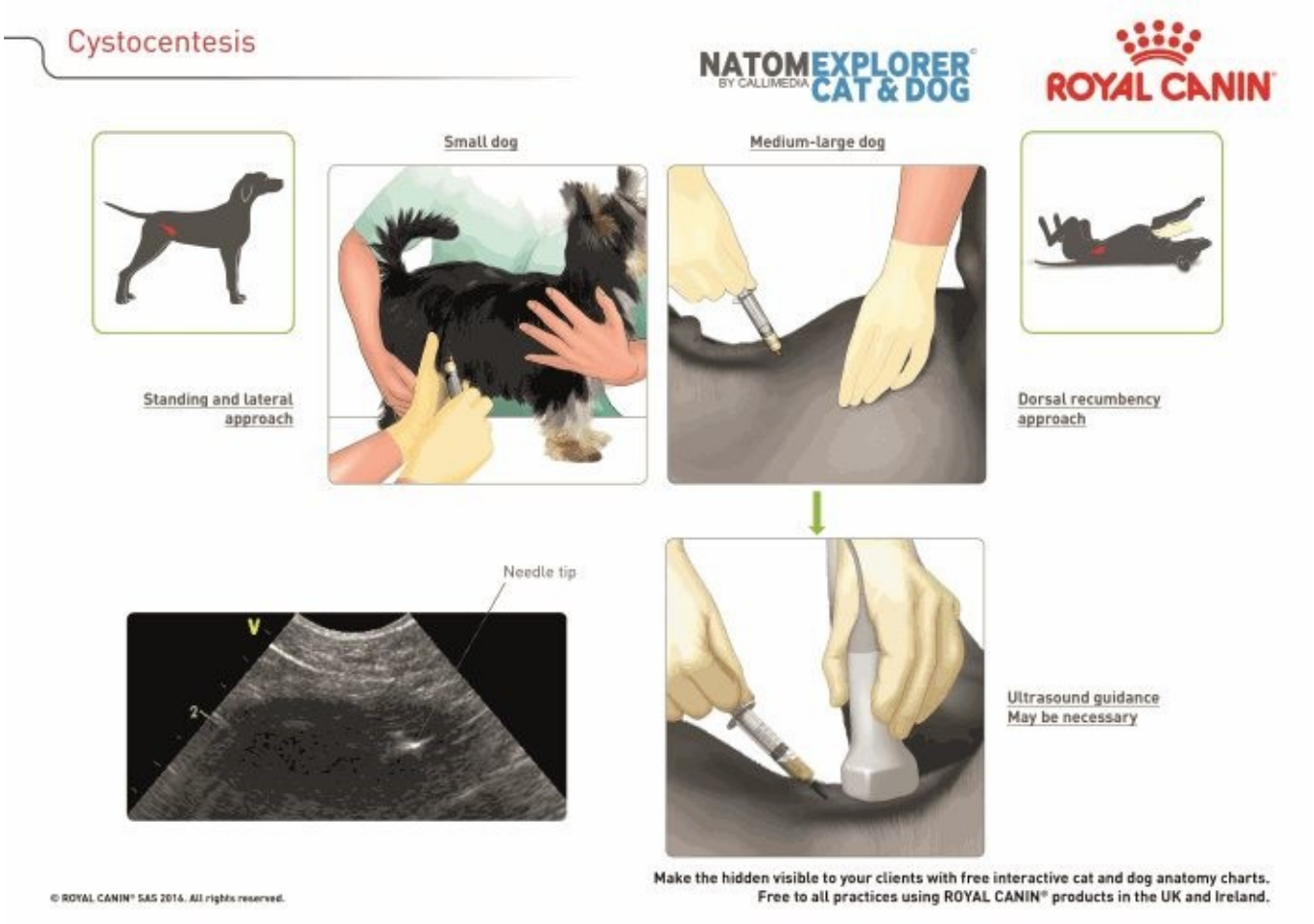
References

- https://www.dpi.nsw.gov.au/animals-and-livestock/animal-welfare/general/general-welfare- of-livestock/sop/cattle/health/urine-collection accessed on 11/06/2018 at 3 PM.
- USAID PREDICT. Marcela Uhart, University of California, Davis (2016) Livestock Sampling Methods: Cattle, Sheep, Goats, Camels, and Swine
- https://pressbooks.umn.edu/cvdl/chapter/module-9-2-collecting-a-sample-to-diagnose-a-

uti/

Annexure:

1. Cystocentesis technique in small animal



SOP No: NCAH/LSU/BIOCHEM 03
Title: SOP on Abdominal or Peritoneal Fluid Collection for Biochemistry Tests
Version No: 1, Total Pages:4
Issue Month/Effective Date: May 2026
Revision Summary: NEW
Supersedes Version No: 1
Prepared by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL,DVL

1. **Scope**

To collect peritoneal (abdominal) fluid aseptically for diagnostic evaluation such as cytology, biochemical analysis, and microbiological testing.

2. **Objective**

To guide the veterinarians and laboratory personnel involved in the diagnosis of abdominal diseases in animals presenting with suspected ascites, peritonitis, abdominal hemorrhage, or organ rupture.

3. **General information/responsibility**

Peritoneal fluid biochemical analysis consist of looking into the variation is the levels of

3.1. Albumin
3.2. Total protein
3.3. WBC & RBC
3.4. Alkaline phosphatase
3.5. Glucose
3.6. Amylase
3.7. LDH (lactate dehydrogenase)
3.8. Cytology
3.9. Bacteriology

4. **Interpretation of results**

4.1. The characteristics of normal peritoneal fluid are clear to slightly yellow with specific gravity less than 1.016 and
4.2. protein (2g/dl, mainly albu- min),
4.3. Total white cell count (2000- 2500/ml, 50% macrophage),some eosinophils, mast cells, and few polymorph neutrophils.

5. **Equipment and Consumables**

5.1. Blood vacuum tubes, 5-10ml: These should be silicone coated, without anticoagulant (red capped).
5.2. Sterile 5-10ml syringe screw-capped, 5ml
5.3. Gloves
5.4. Spirit cotton swab
5.5. Scissor or clipper
5.6. Hypodermic or butterfly needle (21-16G)
5.7. Adaptor
5.8. Serum container

6. **Procedure:**

6.1. Preparation of animal

6.1.1. Properly restrain the animal to minimize movement.
6.1.2. Sedation may be administered if necessary.
6.1.3. Select the puncture site, usually on the ventral midline or slightly lateral to the 2-3 cm midline caudal to the umbilicus or from any four quadrants **(refer annexure 3).**
6.1.4. Clip the hair and clean the area thoroughly.

6.1.5. Disinfect the site using povidone iodine followed by alcohol.

6.2.Sample collection:

6.2.1. Samples are collected from the abdominal cavity from living patients or dead for correlation or as part of clinical diagnosis.

6.2.2. Direct abdominocentesis or USG guided abdominocentesis using 18-20G needle (refer annexure 2) for sample collection

6.2.3. Samples are collected in serum vacutainer (red cap) or EDTA or sterile glass or plastic vial with a stopper.

6.2.4. Dispatch to the lab with a forwarding letter or fill the sample or Laboratory request form (**refer annexure 2).**

7. Safety

The samples should be considered infectious since some of the bacterial pathogens could be zoonotic in nature.

8. Waste Disposal

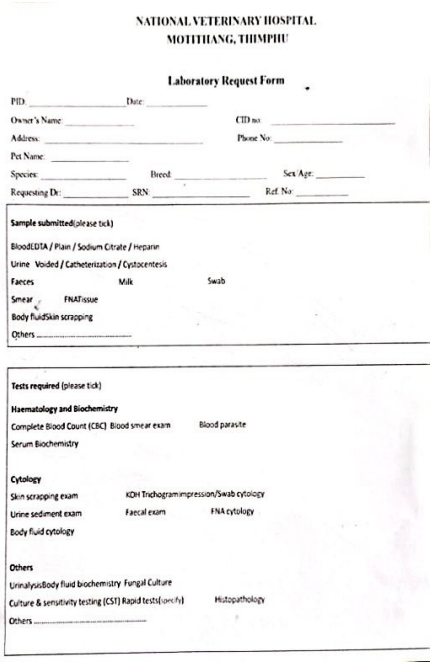
All the waste generated during sample collection should be disposed appropriately based on the zoonotic and non-zoonotic nature of the cases.

9. Reference

1. Mondal, D. B., Kumar, M., Saravanan, M., & Sharma, K. (2012). *Peritoneal fluid analysis in canine disease diagnosis*.
2. https://www.ucsfhealth.org/care/medical-tests/peritoneal-fluid-analysis

Annexure

1. Lab Request form



NATIONAL VETERINARY HOSPITAL
MOTITHANG, THIMPHU

Laboratory Request Form

PID: _____ Date: _____
Owner's Name: _____ CID no: _____
Address: _____ Phone No: _____
Pet Name: _____
Species: _____ Breed: _____ Sex/Age: _____
Requesting Dr: _____ SRN: _____ Ref. No: _____

Sample submitted(please tick)
Blood:EDTA / Plain / Sodium Citrate / Heparin
Urine Voided / Catheterization / Cystocentesis
Faeces Milk Swab
Smear FNA:Tissue
Body fluid:Skin scrapping
Others _____

Tests required (please tick)
Haematology and Biochemistry
Complete Blood Count (CBC) Blood smear exam Blood parasite
Serum Biochemistry

Cytology
Skin scrapping exam KOH Trichogram:impression/Swab cytology
Urine sediment exam Faecal exam FNA cytology
Body fluid cytology

Others
Urinalysis:Body fluid biochemistry Fungal Culture
Culture & sensitivity testing (CST) Rapid tests(specify) Histopathology
Others _____

2. Sites of Abdominocentesis or paracentesis

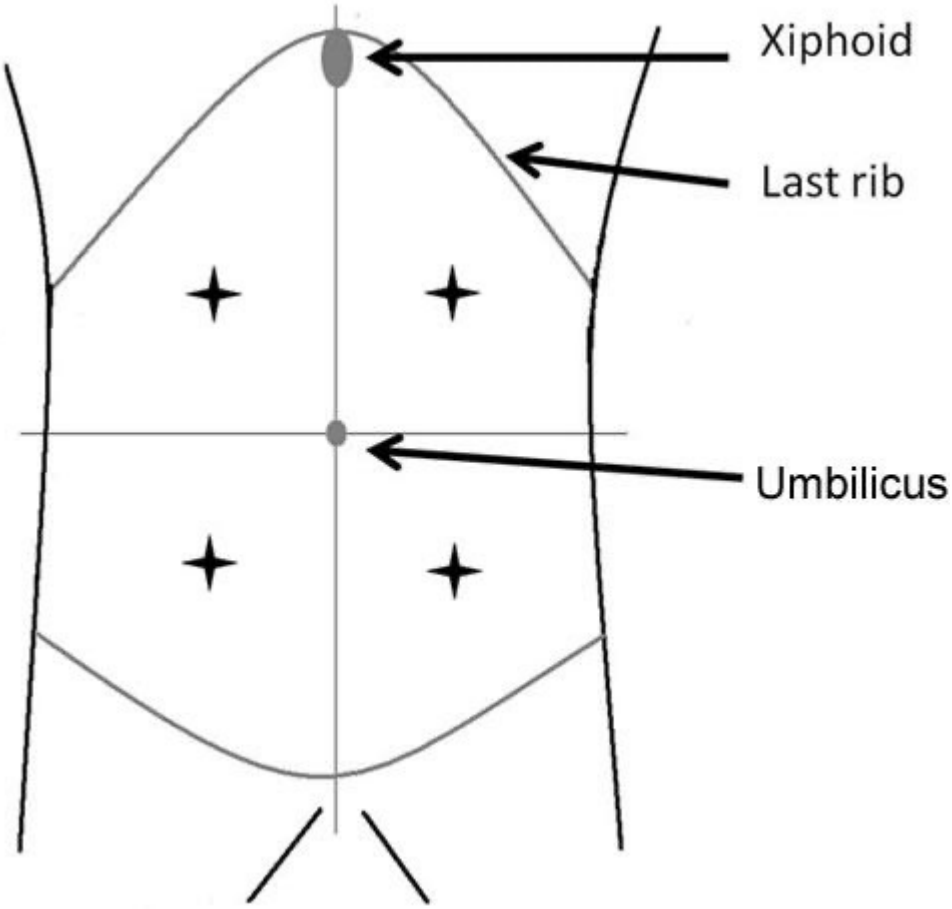


Figure: Diagram showing the sites of needle insertion (+) for standard four-quadrant paracentesis with the patient in dorsal recumbency.

SOP No: NCAH/LSU/BIOCHEM 04
Title: SOP on Quantitative Calcium concentration
Version No: 3, Total Pages:3
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Revision Summary:
Supersedes Version No: 2
Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL, DVL

1. Scope

Calcium is measured to monitor diseases of the bone or calcium regulation disorders. Increased or decreased calcium levels in serum are indicative of various disease conditions.

2. Objective

To describe the procedure of the elisa kit to estimate calcium concentration in serum samples.

3. Principles

Calcium assay kit quantichromtm calcium assay kit (dica-500) is designed to measure calcium directly in biological samples without any pre-treatment. A phenolsulphonephthalein dye in the kit forms a very stable blue coloured complex specifically with free calcium. The intensity of the colour, measured at 612 nm, is directly proportional to the calcium concentration in the sample.

4. Application

Direct assays: ca2+ in serum, urine, saliva etc.

5. Apparatus

a. Pipetting devices and accessories (e.g. 5 µl).
b. Clear bottom 96-well plates (e.g. Corning costar)
c. Plate reader.
d. Cuvettes and spectrophotometer for measuring od612nm.

6. Reagents, solution and buffer

a. Reagent a: 50 ml
b. Reagent b: 50 ml
c. Calcium standard: 1 ml 20 mg/dl ca2+

7. Procedure

Prepare enough working reagent by combining equal volumes of Reagent A and Reagent B. Equilibrate to room temperature before use

a. Procedure using 96-well plate

i. Dilute standards as follows. Transfer 5 µL diluted standards and samples into wells of a clear bottom 96-well plate. Store diluted standards at 4°C for future use.

No	STD + H2O	Vol (µL)	Ca (mg/dL)
1	100µL + 0µL	100	20
2	80µL + 20µL	100	16
3	60µL + 40µL	100	12
4	40µL + 60µL	100	8
5	30µL + 70µL	100	6
6	20µL + 80µL	100	4

7	10µL + 90µL	100	2
8	0µL + 100µL	100	0

ii. Add 200 µL working reagent and tap lightly to mix.
iii. Incubate 3 min at room temperature and read optical density at 570-650nm (peak absorbance at 612nm).

b. Procedure using cuvette

i. Set up test tubes for diluted standards and samples. Transfer 15 µL diluted Standards and samples to appropriately labelled tubes.
ii. Add 1000 µL working reagent and vortex to mix. Incubate 3 min. Transfer to cuvette and read optical density at 612nm.

8. Result and interpretation calculation

Subtract blank od (water, #8) from the standard od values and plot the OD against Ca^{2+} standard concentrations. Determine the slope using linear regression fitting. Calcium concentration of the sample is calculated as

$$= \frac{ODSAMPLE-ODBLANK}{SLOPE} \text{ (mg/dL)}$$

OD **SAMPLE** and OD **BLANK are** OD 612nm values of sample and sample blank (water or buffer in which the sample was diluted).
Conversions: 1 mg/dL Ca^{2+} equals 250 µM, 0.001% or 10 ppm.

9. Waste disposal

Wastes should be disposed as per the sop for disposal of biological wastes and chemicals.

10. Risk assessment

Normal precautions for laboratory reagents should be exercised while using the reagents

11. Troubleshooting

EDTA and other Ca chelators interfere with this assay. This assay cannot be applied to plasma samples obtained with EDTA.

12. References

- Carmela, T.M. et al. (2007). Bactericidal action of daptomycin against stationary-phase and non-dividing *Staphylococcus aureus* cells. Antibacterial Agents and Chemotherapy 51 (12): 4255–4260.
- Hernandez, L. (2007).The antiproliferative role of ERG K+channels in rat osteoblastic cells. Cell Biochem Biophys 47:199–208
- Meggan, E. et al. (2007). Vitamin D receptor-dependent Inhibition of mammary tumor growth by EB1089 and ultraviolet radiation in vivo. Endocrinology 148(10):4887–4894.

SOP No: NCAH/LSU/BIOCHEM 05
Title: SOP on Quantitative Determination of Magnesium
Version No: 3, Total Pages:4
Issue Month/Effective Date: May 2026
Revision Summary:
Supersedes Version No: 2
Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL,DVL

1. Scope

Qualitative colorimetric Magnesium is intended for qualitative determination of magnesium level in serum and urine.

2. Objective

To outline the procedure to measure magnesium concentration in the serum sample.

3. Introduction

Magnesium (mg) is one of the most abundant and essential minerals in mammals. Magnesium is involved in various biochemical reactions in the body and plays important roles in muscle and nerve functions, heart rhythm, and immune system and bone formation. Increase or decrease in the magnesium concentration indicates different disease conditions.

4. Principles

Bioassay systems' magnesium assay kit quanti chromtm magnesium assay kit (dimg- 250) is designed to measure magnesium directly in biological samples without any pre- treatment. A calmagite dye in the kit forms a colored complex specifically with magnesium. The intensity of the color, measured at 500 nm, is directly proportional to the magnesium concentration in the sample. The optimized formulation minimizes interference by potential substances.

5. Apparatus

5.1. Pipettes,
5.2. Spectrophotometer,
5.3. 96 well plates
5.4. Plate reader
5.5. Vortex tube mixer

6. Reagents, solution and buffer

6.1. Reagent a: 25 ml,
6.2. Reagent B: 25 mL,
6.3. EDTA Solution: 2 x 1.5 mL
6.4. Standard: 1 mL 10 mg/dL Mg^2

7. Procedure

Prepare enough working reagent by combining equal volumes of Reagent A and Reagent B. Equilibrate to room temperature before.

b. **Procedure using 96-well plate**

i. Dilute standard to 2 mg/dL by mixing 40 µL 10 mg/dL Standard with 160 µL distilled water. Transfer 5 µL diluted standard and samples in duplicate to wells

of a clear bottom plate. Diluted standard can be stored at 4°C for future use.

ii. Add 200 µL working reagent and tap plate to mix *thoroughly*.

iii. Incubate 2 min at room temperature and read optical density at 500 nm (OD for *sample* and *standard*).

iv. Add 10 µL EDTA Solution to all sample wells and tap plate to mix thoroughly. Incubate 2 min and read OD at 500nm (OD for blanks).

c. Procedure using cuvette

i. Set up test tubes and transfer 25 µL diluted Standard and samples to appropriately labelled tubes.

ii. Add 1000 µL working reagent and vortex to mix. Incubate 2 min.

iii. Transfer to cuvette and read OD500nm. Add 50 µL EDTA solutions, mix well, incubate 2 min and read OD500 nm.

8. Result and interpretation

Magnesium concentration of the sample is calculated as

$$= \frac{OD_{sample} - OD_{blank}}{OD\ Mg - OD\ Mg_{blank}} \times 2\ (mg/dl)$$

OD Sample and OD blank **are** OD500nm values of sample before and after the addition of EDTA. ODMg and ODMg blank are OD500nm values of the standard (2 mg/dL) before and after the addition of EDTA.

Conversions: 1 mg/dL Mg2+ equals 411 µM, 0.001% or 10 ppm.

9. Waste disposal

Wastes should be disposed as per the sop for disposal of biohazard wastes and chemicals.

10. Risk assessment

Normal precautions for laboratory reagents should be exercised while using the reagents

11. Troubleshooting

Edta and other mg2+ chelators interfere with this assay. This assay cannot be applied to plasma samples obtained with EDTA.

Sample pre-treatment: for milk and other lipid/protein-rich samples, mix equal volumes of sample and 10% trichloroacetic a (Sigma Cat# T6399). Incubate 5 min at room temperature and pellet precipitates for 2 min at 14,000 rpm in a table centrifuge. Assay the supernatant (dilution factor = 2) using the above procedure

Sensitive and accurate. Use as little as 5 µL sample. Linear detection.

12. References

- Kim, T. et al (2006). Rapid production of milligram quantities of proteins in a batch cell-free protein synthesis system. J. *Biotechnol*. 124(2): 373-383.

- Pratihar, S. et al (2009). Increased serum magnesium and calcium and their regulation during hibernation in the Indian common toad, Duttaphrynus melanostictus. South Am.
- *J. Herpetology* 4(1): 51-54.
- Stippler, M. et al (2007). Serum and cerebrospinal fluid magnesium in severe traumatic brain injury outcome. *J. Neurotrauma*. 24(8): 1347-1354.

SOP No: NCAH/LSU/BIOCHEM 06
Title: SOP on Quantitative Determination of Phosphorous
Version No: 3, Total Pages:3
Issue Month/Effective Date: May 2026
Revision Summary:
Supersedes Version No: 2
Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL, DVL

1. Scope

Direct assays: phosphorous in serum, urine, saliva etc.

2. Objective

To outline the procedure of the kit to measure phosphorous concentration in serum samples.

3. Introduction

Phosphate (pi) is one of the most important ion species in nature. Phosphate is present in all biological systems. It is one of the important macro minerals in the animals with vital importance. Increased or decreased calcium levels in serum are indicative of various disease conditions.

4. Principles

BioAssay Systems' phosphate assay kit QuantiChromTM Phosphate Assay Kit (DIPI-500)is designed to measure phosphate ions directly in samples without any pre-treatment. The improved Malachite Green method utilizes the malachite green dye and molybdate, which forms a stable colored complex specifically with inorganic phosphate. The intensity of the color, measured at 620nm, is directly proportional to the phosphate concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

5. Apparatus

5.1. Pipetting devices and accessories (e.g. 5 µL).
5.2. Clear bottom 96-well plates (e.g. Corning Costar)
5.3. Plate reader.
5.4. Cuvettes and Spectrophotometer for measuring OD612nm.

6. Reagents, solution and buffer

6.1. Reagent 50 ml
6.2. Pi standard: 14 ml 0.28 mg/dl (30 µm)
6.3. Blank control: 14 ml

7. Procedure

Reagent Preparation:

Important: bring reagents to room temperature and shake before use.

a. Procedure using 96-well plate:

i. Set up standards and samples. Transfer 50 µL distilled water ("Blank"), Standard and samples in duplicate wells of a clear bottom 96-well plate.
ii. Add 100 µL Reagent and tap lightly to mix.
iii. Incubate 30 min at room temperature and read optical density at 620nm (600-660nm).

b. Procedure using cuvette:

i. Set up test tubes labelled Blank, Standard, Samples. Transfer 400µL Water, Standard and samples to appropriately labelled tubes.
ii. Add 800µL Reagent and tap lightly to mix.
iii. Incubate 30 min at room temperature, transfer to cuvette and read optical density at 620 nm (600-660nm).

Important: (1) if sample phosphate concentration is higher than 50 µM dilute samples in distilled water and repeat the assay. (2) It is not necessary to prepare a calibration curve, because the concentration of the provided standard lies within the linear range. (3) Precipitation may occur at high concentrations of phosphate (>100 µM), or in the presence of high concentrations of e.g. proteins and metals. In this case, dilute samples in distilled water and repeat the assay.

8. RESULT AND INTERPRETATION CALCULATION

The phosphate concentration of Sample is calculated as

$$= \frac{OD_{SAMPLE}-OD_{BLANK}}{OD_{SAMPLE}-OD_{BLANK}} \times 0.28 \text{ (mg/dL)}$$

OD**SAMPLE** and OD**BLANK a r e** OD612nm v a l u e s of sample and sample blank Standard and Sample, respectively.
Conversions: 1 mg/dL Pi equals 105.3 µM, 0.001% or 10 ppm.Conversions: 1 mg/dL Ca2+ equals 250 µM, 0.001% or 10 ppm.

9. WASTE DISPOSAL

Wastes should be disposed as per the SOP for disposal of biological wastes and chemicals

10. RISK ASSESSMENT

Normal precautions for laboratory reagents should be exercised while using the reagents

11. TROUBLESHOOTING

- NA

12. REFERENCES

Abranches, J. (2008). CcpA regulates central metabolism and virulence gene expression in Streptococcus mutans. *J Bacteriol.* 190(7):2340-9.

Hildebrand, J. et al (2009) Functional and energetic characterization of P-gp-mediated doxorubicin transport in rainbow trout. Comp Biochem Physiol C Toxicol Pharmacol. 149(1):65-72.

Dunbar, D.R. et al. (2010). Transcriptional and physiological responses to chronic ACTH treatment by the mouse kidney. Physiol *Genomics 40(3): 158-166*

SOP No: NCAH/LSU/BIOCHEM 07
Title: SOP on Quantitative Colorimetric Urea Determination
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Revision: Summary:
Supersedes Version No: 2
Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution:NCAH, NVH, RVH, SVL,DVL

1. Scope

Urea concentration is measured in serum, plasma, urine, milk, cell/tissue culture, broncho-alveolar lavage (BAL) etc.

2. Objective

The objective of the SOP is to describe the procedure to measure the Urea concentration in the sample.

3. Introduction

Urea is the major end product of protein catabolism in animals. It is the primary vehicle for removal of toxic ammonia from the body. Urea produced primarily in the liver are excreted by the kidneys through glomerular filtration with essentially no reabsorption taking place. Measurement of blood urea is often referred as urea nitrogen or Blood Urea Nitrogen (BUN). In general, increased urea levels are associated with various disease conditions of the kidneys.

4. Principles

The improved Jung method utilizes a chromogenic reagent that forms a colored complex specifically with urea. The intensity of the color, measured at 520nm, is directly proportional to the urea concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

5. Application

Urea nitrogen measurements are used in the diagnosis of certain renal and metabolic diseases in conjunction with creatinine measurements. BioAssay Systems' urea assay kit is designed to measure urea directly in biological samples without any pretreatment. Urea concentration is measured in serum, plasma, urine, milk, cell/tissue culture, broncho alveolar lavage (BAL) etc.

6. Apparatus

1. Pipetting devices and accessories (e.g. multi-channel pipette)
2. Clear bottom 96-well plates (e.g. Corning Costar)
3. Plate reader for the plate procedure. Spectrophotometer and cuvettes for measuring OD 510nm or 510nm for the cuvette procedure

7. REAGENTS, SOLUTION AND BUFFER

1.1. Reagent A: 50 mL
1.2. Reagent B: 50 mL
1.3. Urea standard: 1 mL 50 mg/dL

8. Procedure

8.1.Reagent Preparation

8.1.1. Store all components at 2-8°C. For long-term storage, keep standard at – 20°C. Shelf life: 12 months after receipt. Equilibrate reagents to room temperature. Prepare enough working reagent by combining equal volumes of Reagent A and Reagent B, shortly prior to assay. Use working reagent within 20 min after mixing.

8.1.2. 50mg/dL of Urea standard concentration is used for normal BUN assay. However, for low urea samples use 5mg/dL standard and200µL instead of 20µL.

8.2.Procedure using 96-well plate

8.2.1. Serum and plasma samples can be assayed directly (n = 1). Urine samples should be diluted 50-fold in-distilled water prior to assay (n = 50). Transfer 5µ L water (blank), 5µL standard (50mg/dL) and 5µL samples in duplicate into wells of a clear bottom 96-well plate. *For low urea samples (< 5 mg/dL), e.g. issue/cell extract, culture medium, BAL etc, transfer 50 µL water (blank), 50 µL* ***5 mg urea/dL*** *(the 50 mg/dL standard diluted in water) and 50µL samples in duplicate into separate wells.*

8.2.2. Add 200 µL working reagent and tap lightly to mix.

8.2.3. Incubate 20 min (*50 min for low urea samples*) at room temperature.

8.2.4. Read optical density at 520nm. *For low urea samples, read OD at 430nm.*

8.3.Procedure using cuvette

8.3.1. Prepare samples as described for 96-well plate assay. Transfer 20 µL water, standard (50 mg/dL) and samples to appropriately labeled tubes. *For low urea samples, use 5 mg/dL standard and 200µL instead of 20µL.*

8.3.2. Add 1000 µL working reagent and tap lightly to mix.

8.3.3. Incubate 20 min (*50min*) and read OD520nm (*OD430nm*).

9. Result

Urea concentration (mg/dL) of the sample is calculated as

$$[\text{Urea}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{BLANK}}} \times n \times [\text{STD}] \ (mg/dL)$$

OD**SAMPLE**, ODBLANK and OD**STANDARD** are OD values of sample, standard and water, respectively. n is the dilution factor. [STD] = 50 (*or 5 for low urea samples*), urea standard concentration (mg/dL). **Conversions**: **BUN** (mg/dL) = [**Urea]** / 2.14.
1 mg/dL urea equals 167 µM, 0.001% or 10 ppm.
The urea concentration is reported as mg/dL.

10. Waste disposal

Wastes should be disposed as per the SOP for disposal of biohazard materials and chemicals.

RISK ASSESSMENT

N/A

11. Troubleshooting

Some plasma samples will form precipitates after the addition of the working reagent. If this is the case, perform the reaction in a micro tube centrifuge at maximum speed (3000 x g) for five minutes and using the clear supernatant for measurement.

12. References

- Ji, H., Bachmanov, A.A. (2007). Differences in postingestive metabolism of glutamate and glycine between C57BL/6ByJ and 129P3/J mice. Physiol Genomics 31(3):475-82.
- Snykers, S. et al (2007) Chromatin remodeling agent trichostatin A: a key-factor in the hepatic differentiation of human mesenchymal stem cells derived of adult bone marrow. BMC Dev Biol. 7:24.
- Zeng, L. et al (2006). Multipotent adult progenitor cells from swine bone marrow. Stem Cells 24:2355–2366

SOP No: NCAH/LSU/BIOCHEM 08
Title: SOP on Quantitative Estimation of Creatinine
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Revision: Summary:
Supersedes Version No: 2
Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL, DVL

1. Scope

The measurement of creatinine provides an exceptionally useful index of kidney function. BioAssay Systems' creatinine assay kit is designed to measure creatinine directly in biological samples without any pretreatment.

2. Objective

The objective of the SOP is to describe the procedure to measure the creatinine concentration in serum samples.

3. Introduction

Creatinine is synthesized in the body from creatine, which is produced during muscle contractions from creatine phosphate. In the blood, creatinine is removed by filtration through the glomeruli of the kidney and is excreted through urine. In kidney disease, creatinine levels in the blood are elevated, whereas the creatinine clearance rate and hence the urine levels are diminished. Creatinine test is most widely used to assess kidney function.

4. Principle

The improved Jaffe method utilizes picrate that forms a red colored complex with creatinine. The intensity of the color, measured at 510nm, is directly proportional to creatinine concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw sample.

5. Apparatus

5.1. Pipetting devices and accessories (e.g. multi-channel pipette).
5.2. Clear bottom 96-well plates (e.g. Corning Costar).
5.3. Plate reader for the plate procedure. Spectrophotometer and cuvettes for measuring OD 510nm for the cuvette procedure

6. Reagents, solution and buffer

1. Reagent A: 50 mL
2. Reagent B: 50 mL
3. Creatinine Standard: 1 mL 50 mg/dL

7. Procedure

For long-term storage, keep standard at –20°C. Shelf life: 12 months after receipt. Equilibrate reagents to room temperature prior to use. Please note the difference in standard/sample volume and Working Reagent strength for blood and urine assays. This assay is based on a kinetic Jaffe reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. *Use of a multi-channel pipette is recommended.*

1. Procedure using 96-well plate

BLOOD ASSAY (LOW CREATININE LINEAR UP TO 50 mg/dL):

1.1. Dilute standard to 2 mg/dL by mixing 5µL 50mg/dL standard stock and 120µL distilled water. Transfer 30µL diluted standard and serum/plasma in duplicate into wells of a clear bottom 96-well plate.

1.2. Prepare enough Working Reagent by mixing per well reaction at least 100µL Reagent A and 100µL Reagent B. Add 200µL W orking Reagent quickly to all wells. Tap plate briefly to mix.

1.3. Read optical density immediately (OD0) and then at 5 min (OD5) at 490- 530nm (peak absorbance at 510nm).

URINE ASSAY (HIGH CREATININE LINEAR UP TO 300 mg/dL):

1.4. Transfer 5 µL 50 mg/dL standard and urine in duplicate into wells of a clear bottom 96-well plate.

1.5. Prepare enough Working Reagent by mixing per well reaction 50 µL Reagent A, 50µL Reagent B and 100 µL water. Add 200µL Working Reagent *quickly* to all wells. Tap plate briefly to mix.

1.6. Read optical density immediately (OD0) and then at 5 min (OD5) at 490- 530nm (peak absorbance at 510nm).

2. Procedure using cuvette

2.1. Transfer 100µL of 2mg/dL Standard and serum/plasma samples (*Urine Assay: 15*µL 50mg/dL Standard and 15µL urine) to cuvettes.

2.2. Prepare appropriate Working Reagent as above for the 96-well plate procedures. Add 1000µL Working Reagent to each cuvette and pipette *briefly* to mix (avoid bubble formation).

2.3. Read OD immediately (OD0) and at 5 min (OD5) at 490-530nm.

8. Result and interpretation

Creatinine concentration (mg/dL) of the sample is calculated as

$$= \frac{OD_{SAMPLE\ 5} - OD_{SAMPLE\ 0}}{OD_{STANDARD\ 5} - OD_{STANDARD\ 0}} \times n \times [STD]\ (mg/dL)$$

OD**SAMPLE5,** OD**SAMPLE0,** OD**STD5** and OD**STD0** are OD510nm values of sample and standard at 5 and 0 min, respectively. [STD] is 2 mg/dL for blood assay and 50 mg/dL for urine assay.

Conversions: 1 mg/dL creatinine equals 88.4 µM, 0.001% or 10 ppm.
The creatinine concentration is reported as mg/dL**.**

9. Waste disposal

Waste should be disposed as per the SOP for disposal of biohazard wastes and chemicals.

10. Risk assessment

Avoid contact of solution with skin and eyes. Picric acid is an irritant and if dried, potentially explosive. Avoid contact with metals and use large volumes of water during disposal.

11. Troubleshooting

Ascorbic acid, uric acid, glucose, ketones and cephalosporin antibiotics, if present at high concentration concentrations, may interfere in the assay causing falsely high values.

12. References

- Davalos-Misslitz, A.C.M. et al (2007). Generalized multi-organ autoimmunity in CCR7-deficient mice. Eur. J. Immunol. 37: 613–622.
- Wang, J.J. et al (2006). Salutary effect of pigment epithelium– derived factor in diabetic nephropathy evidence for antifibrogenic activities. Diabetes 55: 1678-1685.
- Zhang, S.X. et al (2006). Therapeutic potential of angiostatin in diabetic nephropathy. J Am Soc Nephrol 17: 475–486.

SOP No: NCAH/LSU/BIOCHEM 09
Title: SOP on Glucose tolerance test (Intravenous)
Version No: 3, Total Pages:3
Issue Month/Effective Date: May 2026
Revision: Summary:
Supersedes Version No: 2
Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL, DVL

1. Scope

The test is used to diagnose diabetes in the animals, mostly canines and equines.

2. Objective

The SOP describes the method of conducting Glucose Tolerance Test in animals.

3. Introduction

Normal blood glucose levels vary among species, but individual healthy animals tend to have relatively stable glucose levels because the body's normal homeostatic mechanisms, such as insulin release, maintain glucose within a very narrow range.

Glucose testing is often the first test conducted in diagnosing suspected diabetes. Animals undergoing glucose testing must be properly fasted (8 to 12 hours) before a blood sample is taken because mild, transient hyperglycemia is normal after consuming a meal.

4. Principles

The glucose tolerance test involves administration of glucose followed by serial glucose measurements. The test may be used as part of the initial workup in a patient with suspected diabetes or to aid in determining the therapeutic dose of insulin. In nondiabetic animals, the blood glucose level peaks 30 minutes after administration of glucose and returns to normal within 2 hours, and no glucose appears in the urine. A normal blood glucose level at 2 hours after glucose administration may rule out diabetes mellitus.

5. Apparatus

5.1. IV catheter
5.2. Infusion set
5.3. Vacutainer with anticoagulant

6. Reagents, solution and buffer

6.1. Glucose

7. Procedure

7.1. Fast the animal for 12-16hrs hrs.
7.2. Following the fast, draw a blood sample
7.3. Glucose is administered intravenously to the fasted patient over a 30-second period at a rate of 1.0 g/kg.
7.4. Blood samples are taken at 5, 15, 25, 35, 45, and 60 minutes after glucose infusion, using sodium fluoride as an anticoagulant.
7.5. An additional blood sample is collected after 120 minutes for feline patients. Because of the need for repeated venipuncture, it may be useful to place an IV catheter before the glucose infusion.
7.6. The glucose can be administered via the catheter and blood samples drawn from the catheter.

8. Result and interpretation

The glucose level of each sample is plotted on semi-logarithmic graph paper and the glucose half-life (time required for glucose levels to decrease by 50%) is determined. The post-infusion blood glucose level should decrease to approximately 160 mg/dl in 30 to 60 minutes and return to the baseline value in 120 to 180 minutes. An increased half-life indicates decreased glucose tolerance and suggests diabetes mellitus.

9. Waste disposal

Wastes should be disposed as per the sop for disposal of biohazard wastes and chemicals.

10. Risk assessment

Proper restraining of the animals is required to avoid injury.

11. Troubleshooting

Na

12. References

- http://tnvd.ca/testing-protocols.pml#GLUCOSE accessed on 12/06/2018
- http://www.vetfolio.com/internal-medicine/laboratory-tests-for-diagnosis-and-management- of-diabetes accessed on 12/06/2018

SOP No: NCAH/LSU/BIOCHEM 10
Title: SOP on Automatic Serum analyzer for Biochemistry
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Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL, DVL

1. Scope

This SOP covers the routine operation of the Erba XL-200 Clinical Chemistry Analyzer in the laboratory. It includes preparation, running tests, quality control, and end-of-day procedures.

2. Principle

The Erba XL-200 is an automated clinical chemistry analyzer that measures biochemical substances in blood, serum, plasma, or urine.

2.1. Chemical Reaction:

- The sample reacts with specific reagents, producing a color change proportional to the analyte concentration.

2.2. Photometric Measurement:

The analyzer measures the absorbance of light passing through the reaction mixture. The amount of light absorbed is used to calculate the analyte concentration using **a calibration curve**.

3. Result Reporting:

The analyzer automatically calculates, displays, and stores results.
In short: The Erba XL-200 works by reacting samples with reagents to produce color, measuring light absorbance, and calculating analyte concentration automatically.

4. Materials and Equipment

- Erba XL-200 Clinical Chemistry Analyzer
- Samples (serum, plasma, urine)
- Reagents and calibrators provided for the analyzer
- Quality control materials
- Distilled water and cleaning solutions
- PPE: gloves, lab coat, eye protection
-
-

5. Procedure

5.1. Instrument Preparation

5.1.1. Turn on the analyzer and the connected PC.
5.1.2. Check that distilled water, cleaning solution, and waste containers are filled or empty as required.
5.1.3. Ensure cuvette trays, probes, and sample/reagent racks are in place.
5.1.4. Allow the system to complete automatic warm-up.

5.2. Loading Reagents

5.2.1. Place reagent bottles in the correct positions on the analyzer.
5.2.2. Enter lot numbers and reagent names in the software
5.2.3. Confirm reagents are not expired.

5.3. Loading Samples

5.3.1. Place sample tubes or cups in the sample tray.
5.3.2. Assign each sample to the required test(s) in the software.
5.3.3. STAT (urgent) samples can be loaded in any available position.

5.4.Running Tests

5.4.1. Select the tests or test profiles on the PC.
5.4.2. Review sample assignments, reagent assignments, and QC settings.
5.4.3. Click **Start** to begin analysis.
5.4.4. The analyzer will automatically aspirate, mix, measure, and calculate results.
5.4.5. Review results on the screen and print or export as needed.

5.5.Quality Control;

5.5.1. Run control samples (Normal, High) at the beginning of each batch.
5.5.2. Compare results to expected ranges.
5.5.3. Repeat tests if QC values are outside acceptable limits.

5.6.End-of-Day / Maintenance

5.6.1. Remove all samples, controls, and calibrators.
5.6.2. Run the auto-clean program for cuvettes and probes.
5.6.3. Refill Distilled water and cleaning solutions for the next day.
5.6.4. Wipe down the instrument and surrounding workspace.

6. Result and interpretation

- Review results on the software screen for each patient/sample.
- Print or export results as required.
- Flag any **abnormal values** for supervisor review.
- Record results in the laboratory log or LIMS (Lab Information Management System)
- Compare each test result with **reference ranges**. Which can be **high or low values** and may indicate disease or physiological changes.

7. Waste disposal

Wastes should be disposed as per the sop for disposal of biohazard wastes and chemicals.

8. Risk assessment

The samples (serum, peritoneal fluid, CSF and others) may contain infectious pathogens that may infect humans hence, it should be handled with care.

9. Troubleshooting

Problem	Possible Cause	Recommended Action
Analyzer does not turn on	Power not connected, switch off, or power surge	Check power cable and switch; restart PC and analyzer; verify outlet voltage

PC software does not start	Software error, PC not connected, Windows issue	Restart PC; check USB/ network connection; contact IT support if persistent
Error message: "Probe Error"	Probe blocked or dirty, liquid splash, misalignment	Clean probe assembly; check probe position; run auto-clean; repeat test
Reagent not recognized	Wrong position, barcode unreadable, empty reagent	Place reagent correctly; check barcode; ensure reagent not expired; re-enter in software
Sample aspiration fails	Clogged tip, sample too viscous, tube not seated properly	Check cuvette/sample probe; dilute viscous samples; reseat sample tube
Low or no reaction in test	Reagent expired, wrong reagent, sample degraded	Verify reagent lot and expiry; check sample storage; repeat with correct reagent
Abnormal QC results	Contaminated or degraded controls, incorrect reagent, machine miscalibration	Rerun controls; check reagents; recalibrate if necessary; contact service if persistent
Optical reading error	Cuvette dirty, bubbles in reaction, lamp issue	Clean cuvettes; remove bubbles; check lamp status; run calibration
Error: "Overflow / Waste full"	Waste container full, clogged tubing	Empty waste container; check waste line; run automatic wash
Repeated "System Busy / Frozen"	Software crash, network issues, memory overload	Restart analyzer and PC; close other programs; check software version
Printer does not work	Printer not connected, out of paper, driver error	Check cable/connection; load paper; reinstall driver if needed

Unexpected high/low test values	Sample hemolyzed, lipemic, icteric, or improper handling	Review sample quality; repeat test with fresh sample; flag abnormal results for review

Reference

1. https://www.scribd.com/document/908540366/Operator-Manual-for-XL-200-With-ISE-v2016-02?utm_source=chatgpt.com
2. Erba XL-200 User/Operator Manual (English) – Erba Diagnostics / Erba Lachema

SOP No: NCAH/LSU/BIOCHEM 11
Title: SOP on Biochemical test for Urine or Urinalysis
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Prepared by: Biochemistry, LSU, NCAH
Reviewed by: Dr N.K.Thapa, Dr Karma Choezang, Sonam Wangchuk, Punya Mata, Rinzin Dorji, Thrinang Wangdi,
Approved by:
Application/Distribution: NCAH, NVH, RVH, SVL,DVL

1. **Scope**

To perform urine analysis for the assessment of physical, chemical, and microscopic properties of urine to aid in the diagnosis and monitoring of diseases affecting the urinary system and overall health.

2. **Objective**

The objective of sop is to outline the procedures for urine analysis to identify variations in substances normally found in the urine and to detect substances that should not be present in urine.

3. Introduction

Urine is one of the most easily obtained specimens examined in the laboratory, and examination of the urine not only provides information about the functioning of the kidneys and possible abnormalities of the urinary tract, but may also lead to the diagnosis of various systemic diseases which are reflected by the presence of several substances in the urine.

4. Principles

4.1. Physical Properties

4.1.1. Color

The color of the urine may be any one of the following: colourless, pale yellow, yellow, dark yellow, yellowish brown, greenish yellow, reddish brown, blue milky. The yellow color is due to urochromes. Pus in urine gives a thick color and dark yellow color is due to dehydration as occurs in vomition, diarrhea, serous exudation, fever and in deficient intake of water. In Jaundice, bile pigments are present; the urine is yellow, brown or greenish yellow. The presence of haemoglobin and blood gives the urine a red color.

4.1.2. Transparency

The transparency of the urine is tested by viewing it against light in a test tube. Normally it should be transparent and clear. But in horse, it is cloudy and thick due to the presence of the mucus and calcium carbonate crystals. The horse urine therefore is first allowed to settle, so supernatant is used for other tests. The urine is cloudy if it contains:

a) Epithelial cells in large numbers
b) Leucocytes in large numbers as in pyuria
c) Bacteria in large numbers
d) Mucus as in the urine of the horse
e) Crystals
f) Blood, when present, gives the red or brown smoky appearance.

4.1.3. Odour

The odour of the urine is due to volatile acids. Normal fresh urine has aromatic odour or ammonical odour. Diet, drugs and disease and microorganisms alter the odour of urine. A fresh urine sample having foul odour may indicate urinary tract infection. Strong ammonical odour is observed on standing urine containing bacteria.

4.1.4. **Specific Gravity**

This is a measure of the relative amounts of solids in solution in the urine. Urinary specific gravity provides information about the ability of the kidney to reabsorb water and essential chemicals before they are excreted through urine. Specific gravity is determined by means of Urinometer.

4.1.5. **pH**

The reaction of the urine is dependent on the diet. In herbivores the reaction is alkaline. With carnivores and omnivores it is acidic.

4.2.**Chemical examination**

Chemical examination includes qualitative and quantitative analysis for normal and abnormal constituents and for urinary sediments.

4.2.1. Protein-Robert test and Hellers test

4.2.2. Sugar (Glucose)-Benedict's Test

Urinary sugars when boiled in Benedict's reagent reduce copper sulphate to a reddish cuprous oxide precipitate in hot alkaline medium, the intensity of which is proportional to the amount of sugar present in the urine. The results are reported as I+,2+, etc. depending upon the colour and intensity of the cuprous oxide precipitate.

4.2.3. Ketone (Rothera's Test)

Acetone and acetoacetic acid react with sodium nitroprusside in the presence of alkali to produce a purple colour.

4.2.4. Blood (Benzidine test)

This test is based on peroxidase activity of haemoglobin and its derivatives. Oxygen liberated from Hydrogen peroxide is accepted by Benzidine and the color changes to brown within a few minutes.

4.2.5. Bile Pigment- Gmelin's Test

4.3.**Microscopic examination**

Microscopic examination is done for urinary sediments that may contain cells, casts, crystals, and microorganisms

4.4.**Urine analysis strip:**

A **urine analyzer strip (urine dipstick)** is a quick, semi-quantitative diagnostic tool used to assess multiple biochemical parameters in urine. It can determine all the parameter mentioned above (refer annexure 4)

5. Application

Urinalysis is an important laboratory test that can be readily performed in veterinary practice, and is considered part of a minimum clinical requirement.

It is useful in documenting various types of urinary tract diseases and may provide information about other systemic diseases, such as liver failure and haemolysis.

Microscopic examination of urinary sediments- In healthy animal, the urine contains small numbers of cells and other formed elements from the entire urinary tract, and epithelial cells from the kidney, ureter, bladder, and urethra. In renal disease, the urine often contains increased numbers of substances discharged from an organ that is otherwise accessible only by biopsy or surgery. A microscopic examination of urine sediment detects the presence and amounts of:

- Red blood cells
- White blood cells
- Casts
- Epithelial cells
- Crystals

6. Apparatus

6.1. Chemical examination test:

6.1.1. Test tube
6.1.2. Pipette

6.2. Microscopy Examination of Urinary sediments

6.2.1. Microscope
6.2.2. Centrifuge and its tube
6.2.3. Slide and cover slip

6.3. Urine dipstick

- Analyser strip

7. Reagents, solution and buffer

7.1. Protein

- Robert's Test:

Robert's Reagent- Concentrated nitric acid 1 part, saturated magnesium sulphate 5 parts (prepared by dissolving 770 gm of magnesium sulphate to one litre of water)

- Heller's Test:

Conc. Nitric acid

7.2. Glucose: Benedict 's test

Benedict's qualitative reagent contains: Copper sulphate, glucose reducing agent that converts cupric ions in the copper sulphate to cuprous ion- cuprous oxide.

7.3. Ketone (Rothera's Test)

- Ammonium Sulphate
- 5% sodium nitroprusside
- Strong ammonium hydroxide

7.4. Blood

7.4.1. Benzidine reagent

7.4.2. Glacial acetic acid
7.4.3. Freshly prepared hydrogen peroxide

7.5. **Bile Pigment- Gmelin's Test**
7.5.1. Conc. Nitric acid
7.5.2. Test tube and pipette

7.6. **Microscopy Examination of Urinary sediments**
7.6.1. Distilled water

8. Procedure

8.1. **Protein- Robert's Test**
8.1.1. Take 2 ml of the reagent in a test tube.
8.1.2. With a pipette layer carefully on the reagent, by allowing the urine to flow down the side of the test tube, held slanting, 2 ml of the urine. (note: if the urine is cloudy centrifuge or filter before you do the test)

8.2. **Protein- Heller's Test**
8.2.1. Take 2 ml of conc. Nitric acid in a test tube.
8.2.2. With a dropper or a pipette layer on top, 2ml of urine

8.3. **Glucose- Benedict's test**
8.3.1. Take 5 ml of the reagent in a test tube and heat it.
8.3.2. Add 8 drop of the urine to it and boil it carefully, seeing that it does not spurt while boiling.

8.4. **Ketone Bodies- Rothera's Test**
8.4.1. Ketone bodies consist of Acetoacetic acid, Beta hydroxyl butyric acid and Acetone.
8.4.2. Saturated 5 ml of urine with ammonium sulphate
8.4.3. Add 2-3 drops of 5% sodium nitroprusside solution and then add strong ammonium hydroxide.

8.5. **Blood**
8.5.1. In a test tube dissolve a small quantity of Benzidine in glacial acetic acid.
8.5.2. Add 2 ml of urine to this
8.5.3. Add one ml of freshly hydrogen peroxide and mix.

8.6. **Bile Pigment- Gmelin's Test**
8.6.1. In a test tube take 2ml of conc. Nitric acid
8.6.2. Carefully on top layer 2ml of urine with a pipette

8.7. **Microscopy Examination of Urinary Sediments**
8.7.1. Microscopic examination of urine sediment should be part of a routine urinalysis. For centrifugation, take 3-5 mL of urine in a conical centrifuge tube.

8.7.2. Urine is centrifuged at 1,500-2,000 rpm for ~5 min.
8.7.3. The supernatant is decanted, leaving ~0.5 mL of urine and sediment in the tip of the conical tube.
8.7.4. The sediment is resuspended by tapping the tip of the conical tube against the table several times.
8.7.5. A few drops of the sediment are transferred to a glass slide, and a cover slip is applied.
8.7.6. Examination of unstained urine is recommended for routine samples.

8.7.7. Microscopic examination is performed at 100x (for crystals, casts, and cells) and 400× (for cells and bacteria) magnifications.

Note: methylene blue stain can be used to aid in cell identification but tend to dilute the specimen and introduce artefacts such as stain precipitate and crystals

8.8. **Urine Dipstick Procedure**

8.8.1. Collect urine using midstream catch, catheterization, or cystocentesis into a clean vial.
8.8.2. Dip the strip into the urine for 2–3 seconds, ensuring all reagent pads are fully immersed.
8.8.3. Remove the strip and tap or wipe off excess urine.
8.8.4. Interpret results by either:
- Visually comparing with the color chart, or
- Reading with a compatible urine analyzer.

8.8.5. Before using the analyzer, always perform a control run to verify accuracy.

9. Result and interpretation

9.1. **Protein- Robert's Test**

At the junction of the two fluids a white ring forms if albumin is present.

9.2. **Protein- Heller's Test**

At the Junction of the two liquids a white ring is formed if the urine contains albumin.

9.3. **Glucose- Benedict's test**

If glucose present, a greenish or yellow precipitin forms.

9.4. **Ketone Bodies- Rothera's Test**

A Permanganate colour is indicative of Ketones.

9.5. **Blood**

If blood is present a blue or green colour develops.

9.6. **Bile Pigment- Gmelin's Test**

At the junction of the two fluids a green, violet etc. will be observed if bile pigment is present.

9.7. **Microscopy Examination of Urinary Sediments**

Leukocytes, erythrocytes, and casts may all be of clinical significance when found in urine sediment.

9.7.1. *Leukocytes* - Normally, 0 to 3 leukocytes per high-power field will be seen on microscopic examination. More than 3 cells per high-power field probably indicate disease somewhere in the urinary tract. Estimate the number of leukocytes present per high-power field and report it as the "estimated number per high-power field."

9.7.2. *Erythrocytes* - Red cells are not usually present in normal urine. If erythrocytes are found, estimate their number per high-power field and report it. Erythrocytes may be differentiated from white cells in several ways:

9.7.3. White cells are larger than red cells.

9.7.4. When focusing with the high-power lens, the red cells show a distinct circle; the white cells tend to appear granular with a visible nucleus.

9.7.5. One drop of 5% acetic acid added to the urine sediment disintegrates any red cells, but it does not affect the white cells (except that the nuclei become more distinct).

9.7.6. *Casts - These urinary sediments are formed by coagulation of albuminous material in* the kidney tubules. Casts are cylindrical and vary in diameter. The sides are parallel, and the ends are usually rounded. Casts in the urine always indicate some form of kidney disorder and should always be reported. If casts are present in large numbers, the urine is almost sure to be positive for albumin. There are *seven types* of casts. They are as follows:

9.7.6.1. *Hyaline casts* are the most frequently occurring casts in urine. Hyaline casts can be seen in even the mildest renal disease. They are colorless, homogeneous, transparent, and usually have rounded ends.

9.7.6.2. *Red cell casts* indicate renal haematuria. Red cell casts may appear brown to almost colourless and are usually diagnostic of glomerular disease. White cell casts are present in renal infection and in non- infectious inflammation. The majority of white cells that appear in casts are hyper segmented neutrophils.

9.7.6.3. *Granular casts* almost always indicate significant renal disease. However, granular casts may be present in the urine for a short time following strenuous exercise. Granular casts that contain fine granules may appear grey or pale yellow in color. Granular casts that contain larger coarse granules are darker. These casts often appear black because of the density of the granules.

9.7.6.4. *Epithelial casts* are rarely seen in urine because renal disease that primarily affects the tubules is infrequent. Epithelial casts may be arranged in parallel rows or haphazardly.

9.7.6.5. *Waxy casts* result from the degeneration of granular casts. Waxy casts have been found in patients with severe chronic renal failure, malignant

hypertension, and diabetic disease of the kidney. Waxy casts appear yellow, grey, or colourless. They frequently occur as short, broad casts, with blunt or broken ends, and often have cracked or serrated edges.

9.7.6.6. *Fatty casts* are seen when there is fatty degeneration of the tubular epithelium, as in degenerative tubular disease. Fatty casts also result from lupus and toxic renal poisoning. A typical fatty cast contains both large and small fat droplets. The small fat droplets are yellowish-brown in color.

9.8. **Urine Analyser with dipstick**

Visual Comparison: Reading the dipstick against a color chart provides qualitative and semi-quantitative results.

Analyzer Reading: Inserting the strip into a urine analyzer gives fully quantitative measurements for all parameters.

**Qualitative analysis is also being done by rapid method using strips which is dipped in the urine samples and colour changes are being matched against each color coded agents as described by the manufacturer.*

10. Waste disposal

Waste should be disposed as per the sops for disposal of biohazard wastes and chemicals.

11. Risk assessment

Avoid contact of solution/reagents with skin and eyes.

12. Troubleshooting

12.1. Collection method may impact microscopic (and bacteriologic) findings and interpretation.

6. For example, cystocentesis samples may contain RBCs
7. Catheterized samples may contain lubricant gel and epithelial cells
8. Voided samples may contain debris and epithelial cells and may be contaminated with bacteria.

12.2. Hence, always record the method of urine collection so the results can be interpreted accurately. A concentrated sample (first urine in the morning) is best.

12.3. Urine is unstable and must be analyzed promptly! It should be collected in clean or sterile containers and if not analyzed within a short period of time (e.g., an hour or two), it should be refrigerated. Cold urine should be allowed to return to room temperature prior to analysis to avoid a false increase in specific gravity.

12.4. Precipitates may form in urine as it cools, and cold may interfere with some chemical tests. Urine collected for some biochemical tests (e.g., electrolytes) may be frozen. If mailing specimens, check first with the laboratory to check on recommended preservatives.

12.5. Effects of delayed analysis include bacterial overgrowth, increased pH, formation or dissolution of crystals, breakdown of casts, lysis of erythrocytes, and breakdown of bilirubin.

12.6. **Rothera's Test:** Normal urine does not contain methyl ketone. Weak false positive

reactions may occur if the urine contains L-dopa and phenyl pyruvic acid. If there is suspicion of a false positive test, heat the urine in a test tube in a Bunsen burner flame for one minute, allow cooling and repeating the Rothera's test. Heated urine will not give a positive Rothera's due to ketone bodies.

12.7. **Benedict's test:** Normal urine does not contain any reducing sugar. If protein is present in large amounts, it may interfere with the precipitation of the cuprous oxide. To overcome this problem, precipitate the proteins using 3% SSA filter using a Whatman filter paper and use the filtrate to test the amount of sugar present.

13. References

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14. Appendix

Haemoglobin cast

TEST CATEGORIZATION BIO-CHEMISTRY

Sl. No.	Procedure / SOP	DVL	SVL/ TVH	RLDC/ NVH	NCAH
1	Serum Bio-chemistry Mineral estimation (macro/micro) 1. Calcium 2. Magnesium 3. BUN 4. Creatinine			X	X

2	1. Qualitative urine analysis 2. Colour, 3. RBC, 4. Leukocytes, 5. Nitrite, 6. casts, 7. epithelial cells, 8. transparency, 9. bile pigments, 10. protein, 11. sugar, 12. ketone bodies, 13. blood 14. pH, specific gravity,	X	X	X	X
3	Quantitative bio-chemistry 1. BUN 2. Creatinine 3. Glucose tolerance			X	X