Scientific Works. Series C. Veterinary Medicine. Vol. LXIV (1), 2018
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

CURRENT KNOWLEDGE ON URINE ELECTROPHORESIS IN CLINICAL MEDICINE

Ana Maria GOANȚĂ[1], Roxana IGNĂTESCU (ȚÎMPĂU)[1], Ana Maria Alexandra STĂNESCU[2], Valentina Alina SIMION[1], Lucian IONIȚĂ[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Carol Davila University of Medicine and Pharmacy, 37 Dionisie Lupu Street, District 2, Bucharest, Romania

Corresponding author email: ana_mv@yahoo.com

Abstract

Electrophoresis defines the migration of charged particles in an electrical field in accordance to their molecular charge and size. In clinical medicine, electrophoresis is used mainly to separate and thus differentiate between and proteins in a given sample, be it serum, urine, cerebrospinal fluid or others. This paper aims to briefly describe the fundamentals and scope of electrophoresis and review the most recent knowledge on urine protein electrophoresis. Urine electrophoresis (UEP) is always evaluated in conjunction with serum electrophoresis and a measurement of total urine protein. In human medicine, proteinuria has been identified and characterised based on this criterion. UEP can also be used to differentiate between glomerular and tubular disease, based on the quantity and size of the molecules. Considering the advances in human medicine and the wealth of disorders that can present with proteinuria in animals, the authors consider that this diagnostic technique deserves more attention in veterinary medicine, in particular as a valuable aid in the detection and identification of renal lesions.

Key words*: proteinuria, electrophoresis, urine.*

INTRODUCTION

Proteinuria is defined as the presence of abnormal quantities of protein in the urine. In healthy animals, proteins that pass through a glomerulus are reabsorbed by the renal tubules or broken down by renal tubular epithelial cells (Harley L and Langston C, 2012). The characteristics of the molecule of protein (size, shape, and charge) determines its ability to pass through the glomerular filter (Latimer KS, 2011). The types of protein normally found in human urine are plasma proteins (30% albumin, 30% serum globulins) and around 40% are tissue proteins from the kidney and urinary tract (mainly Tamm–Horsfall protein, a glycosylated product from the loop of Henle, alongside protein from epithelial cells, casts or exostomes (Wein et al., 2016, Keren DF, 2003, Barrat et al, 2007; Kalantari S et al, 2013). Determining the composition of proteinuria is a non-invasive diagnostic approach that helps distinguish different disease processes. The three mechanisms that result in proteinuria are glomerular injury (with excessive filtration of protein); tubular injury (with excessive production and excretion of tubular proteins) and overfiltration of plasma protein in hyperproteinemia (Toto RD, 2004). The main causes of proteinuria in small animals are summarised in Table 1. In human medicine, clinical proteinuria (>0.5 g/24h) is recognized as the strongest risk predictor of progression to end-stage renal failure and a strong predictor of risk of cardiovascular disease (Camaré C and Caussé E, 2013; Johnson DW, 2011).

In recent years, urinary proteomics has garnered a lot of interest due to its advantages: urine can easily obtained in large quantities and without the need for qualified personnel; the protein it contains is relatively stable; its protein content is less complex and requires less processing than that of blood, which facilitates analysis and interpretation and it usually contains few cells, lipids and less soluble protein that can interfere with analysis (Mischak et al., 2010).

Determining and analysing proteinuria can be done in a multitude of ways. The most frequent is point-of-care testing using urine reagent

strips (Figure 1) in various pathologies such as diabetes, hypertension, systemic diseases, renal disease in order to monitor the effect of nephrotoxic drugs. The dipstick test is most sensitive for albumin but can detect other proteins (Harley L and Langston C, 2012) and it can be combined with the sulfosalicylic acid (SSA) precipitation test which detects all types of protein (Guedes-Marques M. et al, 2015). If the SSA test is positive but urine dipstick is negative, UEP is required to evidentiate immunoglobulin light chain excretion due to dysproteinemias (Patel VB and Preedy VR, 2016). Herbivores that produce a highly alkaline urine always obtain a false-positive reaction for proteinuria (trace or 1+) on urine dipstick tests (Constable et al., 2017). Recently, microalbuminuria assays have been recommended in both human and veterinary medicine as screening tests, as they are more sensitive than the dipstick and sulfosalicylic acid methods for albumin (Bartges J and Polzin DJ, 2011; Rangaswami J et al., 2017).

Specification

	Measurement principle
GLU	Glucose oxidase reaction
PRO	Protein-error reaction
BIL	Azo-coupling reaction
URO	Azo-coupling reaction
pH	pH indicator
S.G.	Cation extraction
BLD	Activity measurement of pseudoperoxidase in hemoglobin
BLD(10PA)	Activity measurement of pseudoperoxidase in hemoglobin
KET	Sodium nitroprusside method
NIT	Grease reaction
LEU	Activity measurement of esterase in leukocytes
CRE	Chelate competition method

	Measurement range	Visual measurement time
GLU	50-1000 mg/dl	60 sec.
PRO	15-1000 mg/dl	60 sec.
BIL	0.5-6.0 mg/dl	60 sec.
URO	2-8 mg/dl	60 sec.
pH	pH 5-9	60 sec.
S.G.	S.G. 1.000-1.030	60 sec.
BLD	hemoglobin 0.06-1.0 mg/dl	60 sec.
BLD(10PA)	hemoglobin 0.03-1.0 mg/dl	60 sec.
KET	5-150 mg/dl	60 sec.
NIT	nitrite 0.08-0.5 mg/dl	60 sec.
LEU	25-500 Leu/μl	90 sec.
CRE	10-300 mg/dl	60 sec.

Fig. 1. An example of a urine reagent test strip commonly used in veterinary medicine in Romania. GLU glucose, PRO total protein, BIL bilirubin, URO urobilinogen, S.G. specific gravity, BLD blood, KET ketones, NIT nitrite, LEU leukocytes, CRE creatinine (ARKRAY Europe, B.V.)

Due to the frequent occurence of false positive and false negative reactions, it is highly recommended to quantify and type proteinuria in a laboratory. This can be achieved through quantitative measurements with devices using various reactions (the biuret method or colorimetry) and through qualitative quantifications though various various techniques: electrophoretic methods or immunochemistry (Camaré C and Caussé E, 2013).

The urine protein to creatinine ratio (UPCR) obtained by dividing the urinary protein concentration (mg/dL) by the creatinine concentration (mg/dL) is a ratio that serves as a sensitive and reliable method to detect and quantify proteinuria (in samples that present no inactive sediment) and can replace the protein count in a 24-hour sample (Constable et al, 2017). In healthy dogs UPCR is usually <0.5 and in cats it is <0.4 (Harley L & Langston C, 2012). The normal UPCR in the horse is considered less than 1.0 (Constable et al., 2017). Values over 0.5 in dogs and cats are considered proteinuric; values > 1.0 are considered pathologic and glomerular proteinuria is usually associated with a ratio of over 2.0 (Duffy et al, 2015; Lees et al, 2004, Harley L and Langston C, 2012). In horses with proteinuria, UPCR under 13 is believed to be indicative of tubular proteinuria (Constable et al., 2017). Urine protein electrophoresis can be performed on a spot urine sample (morning preferable) or a 24-hour collection (Lee et al 2017). While serum is processed undiluted, UEP requires concentration and desalting before processing (Keren DF, 2003). For electrophoresis, urine (either from one miction or from a 24-hour urine collection) is centrifuged to eliminate the mineral and organic sediment and analysed immediately or stored at -80°C (Magdeldin et al., 2012).

Electrophoresis defines the migration of charged particles in an electrical field in accordance to their molecular charge and size and can differentiate mixed populations of protein macromolecules into large protein fractions such as albumin, alpha-1, alpha-2, beta and gamma globulins and even into subunit structures such as polypeptides that differ by a few hundred daltons or 0.1 pH units, depending on the techniques and equipment available (Westermeier R, 2016).

Table 1. Differential diagnosis for small animal proteinuria, from Gough A, Murphy K, Differential Diagnosis in Small Animal Medicine, 2nd Edition, Wiley Blackwell, 2015; modified with data from Harley L and Langston C, 2012

Proteinuria
False positives (strip test)
Contamination, e.g. benzalkonium chloride, cetrimide, chlorhexidine
Stale urine
Highly alkaline urine (pH greater than 8.0)
False positives (20% sulphosalicylic acid test)
Cephalosporins
Penicillins
Radiographic contrast media
Sulphafurazole
Thymol
Tolbutamide
Pre-renal
Fever, heat stroke
Central nervous system disease (eg. seizures)
Pancreatitis
Systemic hypertension; cardiac disease (eg. CHF)
Drug reactions
Acute pancreatitis
Hyperthyroidism (cat)
Hyperadrenocortism (dog)
Haemoglobinuria, e.g. haemolytic anaemia
Hyperproteinaemia e.g. derived from colostral proteins, monoclonal free light chain (multiple myeloma)
Myoglobinuria, e.g. muscle trauma, rhabdomyolysis
Physiological, e.g. exercise, stress
Renal
Mild to moderate
• Acute kidney injury
• Amyloidosis
• Breed-associated nephropathy (dog)
• Chronic kidney disease
• Fanconi syndrome
• Glomerulonephritis
• IgA nephropathy
• Primary renal glucosuria
• Secondary glomerular disease
• Bacterial endocarditis
• Borreliosis
• Brucellosis
• Chronic bacterial infection
• Chronic skin disease
• Diabetic glomerulosclerosis
• Dirofilariasis
• Ehrlichiosis
• Exogenous steroid use
• Feline infectious peritonitis (cat)
• Feline leukaemia virus (cat)
• Hyperthermia
• Hypothermia
• Immune-mediated hemolytic anemia
• Infectious canine hepatitis (dog)
• Inflammatory bowel disease
• Leishmaniasis
• Leptospirosis
• Mycoplasma polyarthritis
• Pancreatitis
• Polyarthritis
• Prostatitis
• Pyometra
• Pyrexia
• Rocky Mountain spotted fever (dog)
• Septicaemia
• Sulphonamide hypersensitivity
• Systemic lupus erythematosus
Severe:
• Amyloidosis
• Glomerulonephritis
Post-renal
Genital tract disease: prostatitis, vaginitis
Genital tract secretions
Lower urinary tract disease: trauma, urinary tract infection, urolithiasis
Urogenital neoplasia: bladder, ureteral, urethral, vaginal or prostatic neoplasia

Electrophoretic techniques commonly used are horizontal and vertical gel electrophoresis, agarose gel electrophoresis, polyacrylamide gels, sodium dodecyl sulphate-polyacrylamide gel electrophoresis, native (buffer) gels, gradient gels, capillary electrophoresis, cellulose acetate electrophoresis, isoelectric focusing and two-dimensional gel electrophoresis, microchip electrophoresis. Several techniques can be used in conjunction with electrophoresis, such as immunofixation, immunonephelometry or mass spectrometry (Keren, 2003; Kurien BT and Scofield RH, 2012, Westermeier R, 2016).

In human clinical medicine, urinary protein electrophoresis is used by nephrologists and cardiologists to look for signs of glomerular or tubular proteinuria, while hematologists look for paraprotein suggestive of malignant hemopathies (Camaré C and Caussé E, 2013). UEP can help distinguish if the proteinuria is glomerular or tubular (Jenkins MA, 2009). However, urinary protein electrophoresis is a semi-quantitative method that has poor performance for the study and monitoring of proteinuria and in order to obtain more information one should investigate glomerular and tubular urinary markers such as IgG, albumin, transferrin, alpha-1-microglobulin and retinol binding protein using complementary methods such as immunonephelometry (Bastard JP et al., 2017). Nephelometry is the

measurement of scattered light used to determine the size, shape, and concentration of scattering particles; in immunoassays, these particles are the antigen-antibody complexes formed (Ackerman E, Rosevear JW, 1979). Immunoelectrophoresis includes a variety of techniques that combine electrophoresis and the precipitation reaction between antibody and antigen (Csako G in Kurien BT and Scofield RH, 2012). Immunofixation electrophoresis is an alternative to immunoelectrophoresis that helps identify specific proteins in situ (Csako G in Kurien BT and Scofield RH, 2012).

MATERIALS AND METHODS

In order to review current knowledge on urinary electrophoresis in human and veterinary medicine we searched through scientific databases using keywords such as 'urinary', 'electrophoresis', 'veterinary', 'small animal', 'large animal'. We selected those results that were relevant to the topic and presented both well established and novel uses for UEP.

RESULTS AND DISCUSSIONS

The main reason for performing UEP is to monitor and diagnose monoclonal gammopathies, which are disorders in which lymphocytes/plasma cells proliferate and produce monoclonal intact immunoglobulin (IgG, IgA or IgM), free light chain or free heavy chain protein (Tate et al, 2009; Jenkins, 2009), resulting in different electrophoretic patterns, as in chronic lymphocytic leukemia and multiple myeloma (Keren DF, 2003). In small animals, serum monoclonal gammopathies can be caused by canine ehrlichiosis, leishmaniasis, pyoderma, B‐CLL, B‐cell lymphoma, plasmacytoma and feline infectious peritonitis (FIP), and some of these protein can also be found in urine, as in myeloma or FIP (Harley and Langston, 2012; Meuten DJ, 2017). Further investigations are required to identify the type of protein involved and the cause, thus determine the severity of the lesion - benign monoclonal gammopathy, multiple myeloma or light chain amyloidosis (Tate et al, 2009; Jenkins, 2009).

Routine biochemistry in an animal may reveal a low albumin to globulin ratio, which should be followed by serum and urine electrophoresis. A decreased albumin to globulin ratio is either due to renal proteinuria and/or excessive immunoglobulin production due to antigenic stimulation or a monoclonal gammopathy (Latimer KS, 2011). B-cell chronic lymphocytic leukemia (CLL) in humans is accompanied by macroglobulinemia; in animals this is more rare and, if present, the globulin is usually IgM (identified through immunoelectrophoresis) (Meuten DJ, 2017). Electrophoresis can thus help distinguish between a T-cell or B-cell CLL.

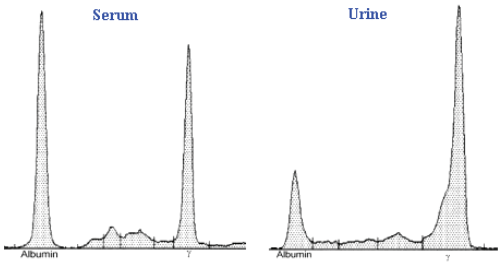

Fig. 2. An example of serum and urine electrophoretograms from a cat with multiple myeloma - a monoclonal peak in the γ region can be observed in both serum and urine electrophoretograms; the immunoglobulin was IgG (Cornell University Veterinary Diagnostic Laboratory)

In myeloma, one usually encounters a mono- or biclonal gammopathy, most frequently constituted of IgG, less so IgA and IgM, while some are nonsecretory (Meuten, 2017). These gammopathies can be identified in the serum, while in urine one can identify light chain immunoglobulins (Fig. 2) that are small enough to pass through the nephron; these globulins can determine glomerulonephritis or nephrotic syndrome (Meuten DJ, 2017). The diagnosis of B-cell or plasma cell lymphoma can be aided by UEP through the detection of large amounts of monoclonal immunoglobulin light-chain when renal damage can be excluded from the differential (Schwab M, 2011).

UEP is also useful for investigating proteinuria of renal origin and distinguishing between glomerular and tubular protein (Fig. 3). The glomerular filter is composed of three successive barries: the endothelial cells, basement membrane and podocyte foot processes that work together (Bartges J, Polzin DJ, 2011; D'Amico G, Bazzi C 2003). In

glomerular kidney disease, UEP will identify albumin as the cause of proteinuria, as larger protein molecules can be lost through urine (Lee et al, 2017; Wein et al., 2016). Tubular lesions impair the reabsorbtion of low molecular weight proteins, with increases in alpha-1 and beta-2 fractions in urine such as retinol binding protein and alpha-1 microglobulin (Jenkins, 2009). Tubular damage may result in high molecular weight protein loss in the urine, which must be differentiated though UEP immunofixation to determine its nature and origin (Lee et al., 2017; Wein et al., 2016). Yalçin A and Çetin M (2004) used UEP and imunoblotting to identify transferrin, alpha-1 microglobulin, beta-2-microglobulin and retinol binding protein (RBP) in dogs with renal disease and detected RBP in all patients with proteinuria and in two healthy dogs. Schaefer et al. (2010) also detected RBP in dogs with various pathologies diagnosed with systemic inflammatory response syndrome. Several studies identify RBP as a possible biomarker in human as well as animal kidney disease (Nabity et al., 2011). In small animal medicine, it is important to note that renal disease can be present in the absence of increased blood urea nitrogen or creatinine values and proteinuria is a negative prognostic factor in renal disease (Harley L and Langston C, 2012). On 49 dogs admitted for an increase in serum creatinine, proteinuria or both, Zini et al. (2004) performed renal biopsy and histologic examination as well as urinalysis and SDS-agarose gel electrophoresis on urine collected through cystocentesis and compared the histopathologic score with the electrophoretic pattern. They observed that dogs with glomerular disease has very similar proteinuric patterns and cannot be differentiated on this basis (Zini et al., 2004). When tubular and tubulo-interstitial lesions were detected on histology, only the most severe forms were accompanied by a tubular pattern on the urinary electrophoretogram, and the smaller the molecules detected, the more severe was the histological score (Zini et al., 2004). It is recommeded that proteinuria be treated if it persists after the inciting causes have been managed, in particular in the presence of polyuria and polydypsia (Harley L and Langston C, 2012) - see Figure 4.

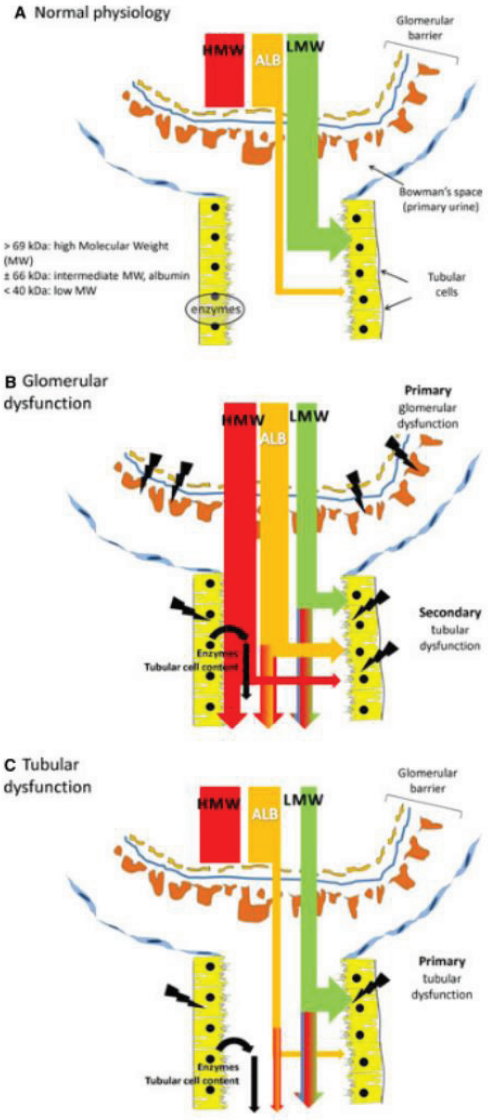

Fig. 3. Nephron filtration of plasma protein: A = physiologic; B = primary glomerular dysfunction with secondary tubular dysfunction; C = primary tubular dysfunction. In health, a small quantity of low molecular weight protein and albumin pass through the glomerular barrier to be reabsorbed by tubular cells. When different disease processes alter the permeability of the glomerular membrane and/or overcome the reabsorbtive capacity of the tubular cells, urine may contain albumin (ALB), low molecular weight protein (LMW) and high molecular weight protein (HMW) (Reproduced from de Loor et al., 2013).

Nephrotic syndrome, defined as the presence of proteinuria, hypoalbuminemia, extravascular accumulation of fluids and hyperlipidemia, is an uncommon occurence in dogs and cats with

protein-losing nephropathy (Bartges J, Polzin DJ, 2011). It presents with decreased serum albumin and γ-globulin and increased α2-albumin, while UEP demonstrates increased albumin and some increase in globulins (Keren, 2003; Longsworth LG, MacInnes DA, 1940). Nephrotic syndrome can be more readily diagnosed and its cause discovered through a complex urine examination (routine urinalysis, UEP and immunofixation), as the serum electrophoretic pattern may only become evident in severe disease (Keren 2003).

Anti-proteinuric drugs (body weight–BW)

	Feline dose	Canine dose
ACE inhibitors		
— Enalapril[a]	0.25 to 0.5 mg/kg BW, PO, q12 to 24h	0.5 mg/kg BW, PO, q12 to 24h
— Benazapril[a]	0.25 to 0.5 mg/kg BW, PO, q12 to 24h	0.25 mg/kg BW, PO, q12h
— Lisinopril[a]	0.25 to 0.5 mg/kg BW, PO, q12 to 24h	0.25 to 0.5 mg/kg BW, PO, q12 to 24h
Angiotensin II receptor antagonists		
— Losartan[b] — Telmisartan[b]	No data	0.5 to 1 mg/kg BW/d
Aldosterone receptor antagonist		
— Spironolactone[a]	0.5 to 1 mg/kg BW/d	0.5 to 1 mg/kg BW/day
Omega 3 Fish Oil[b]	Minimum of 1 g/4.55 kg BW, q24h	Minimum of 1 g/4.55 kg BW, q24h
Antihypertensive drugs (if animal persistently hypertensive despite ACE inhibitor): e.g., Amlodipine[a]	0.2 to 0.4 mg/kg BW, q12h	0.2 to 0.4 mg/kg BW, q12h

[a] Plumb DC. Veterinary Drug Handbook, 7th ed. Ames, Iowa: Wiley-Blackwell, 2011.
[b] No published dose in dogs and cats. Dose extrapolated from human data and suggested dose based on authors' experience.

Fig. 4. Antiproteinuric drugs (from Harley L and Langston C, 2012)

UEP is currently used in the search for protein biomarkers in specific diseases in order to monitor and detect diseases in earlier stages. To this end, the most common EP technique employed is capillary electrophoresis-mass spectrometry (CE-MS), which offers both high separation efficiency and molecular mass information (Shao C et al, 2011). Panels of proteins and peptides that may facilitate earlier detection of diseases such as acute kidney injury, diabetic nephropathy, glomerular diseases and distinguish between bladder, kidney, prostate cancer and other genito-urinary diseases have already been identified (Shao C et al, 2011, Siwy J et al., 2017).

Researchers are also looking at the urine proteome for early markers of non-renal disease such as acute pancreatitis, obstructive sleep apnea and non-small-cell lung cancer, among others (Barrat J, Topham P, 2007; Shao C et al 2011). Nally JE et al. (2015) devised a method to detect urinary biomarkers of chronic infection in clinically asymptomatic and serologically negative rats with leptospirosis, the most common reservoir hosts (Nally JE et al., 2015). Song et al. (2013) devised a method for the rapid detection of bacteria in urine using capillary electrophoresis with a limit of detection of 106 CFU/mL. Urine capillary electrophoresis can be used alongide complementary methods for the metabolic profiling of urine, rapid screening for drug abuse, toxic compounds and metabolites (Kohler I et al, 2013; Zhang Q et al., 2015; Wang W et al., 2010) alongside other techniques.

CONCLUSIONS

UEP is essential to determining the profile of the urinary proteome. Urine is an easily sampled biological fluid that is being searched worldwide for biomarkers of disease, both of the genito-urinary tract and of other systems. It is also useful to detect subclinical infection, toxic compounds in humans, pets and large animals or growth promoters in food-producing animals. Urine proteomics in animals and UEP in particular are not well established at the moment. We consider that serum electrophoresis and UEP should become routine investigations in animals with relevant clinical signs and for monitoring purposes and that a minimal urinalysis (dipstick and sediment) should be included in all routine examinations.

ACKNOWLEDGEMENTS

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**** Cornell University College of Veterinary Medicine, New York State Veterinary Diagnostic Laboratory; <https://ahdc.vet.cornell.edu/images/ClinPath/test/immun/urineelp.gif>