



# Dried Blood Spots technology for veterinary applications and biological investigations: technical aspects, retrospective analysis, ongoing status and future perspectives

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## Abstract

Dried Blood Spots (DBS) technology has become a valuable tool in medical studies, however, in veterinary and biological research DBS technology applications are still limited. Up-to-date no review has comprehensively integrated all the evidence existing across the fields, technologies and animal species. In this paper we summarize the current applications of DBS technology in the mentioned areas, and provide a scope of different types of dried sample carriers (cellulose and non-cellulose), sampling devices, applicable methods for analyte extraction and detection. Mammals, birds, insects and other species are represented as the study objects. Besides the blood, the review considers a variety of specimens, such as milk, saliva, tissue samples and others. The main applications of dried samples highlighted in the review include epidemiological surveys and monitoring for infections agents or specific antibodies for disease/vaccination control in households and wildlife. Besides the genetic investigations, the paper describes detection of environmental contaminants, pregnancy diagnosis and many other useful applications of animal dried samples. The paper also analyses dried sample stability and storage conditions for antibodies, viruses and other substances. Finally, recent developments and future research for DBS technology in veterinary medicine and biological sciences are discussed.

**Keywords** Dried blood spots · Dried matrix spots · Veterinary diagnostics · Epidemiological surveys · Sero-surveillance · Genetic investigations

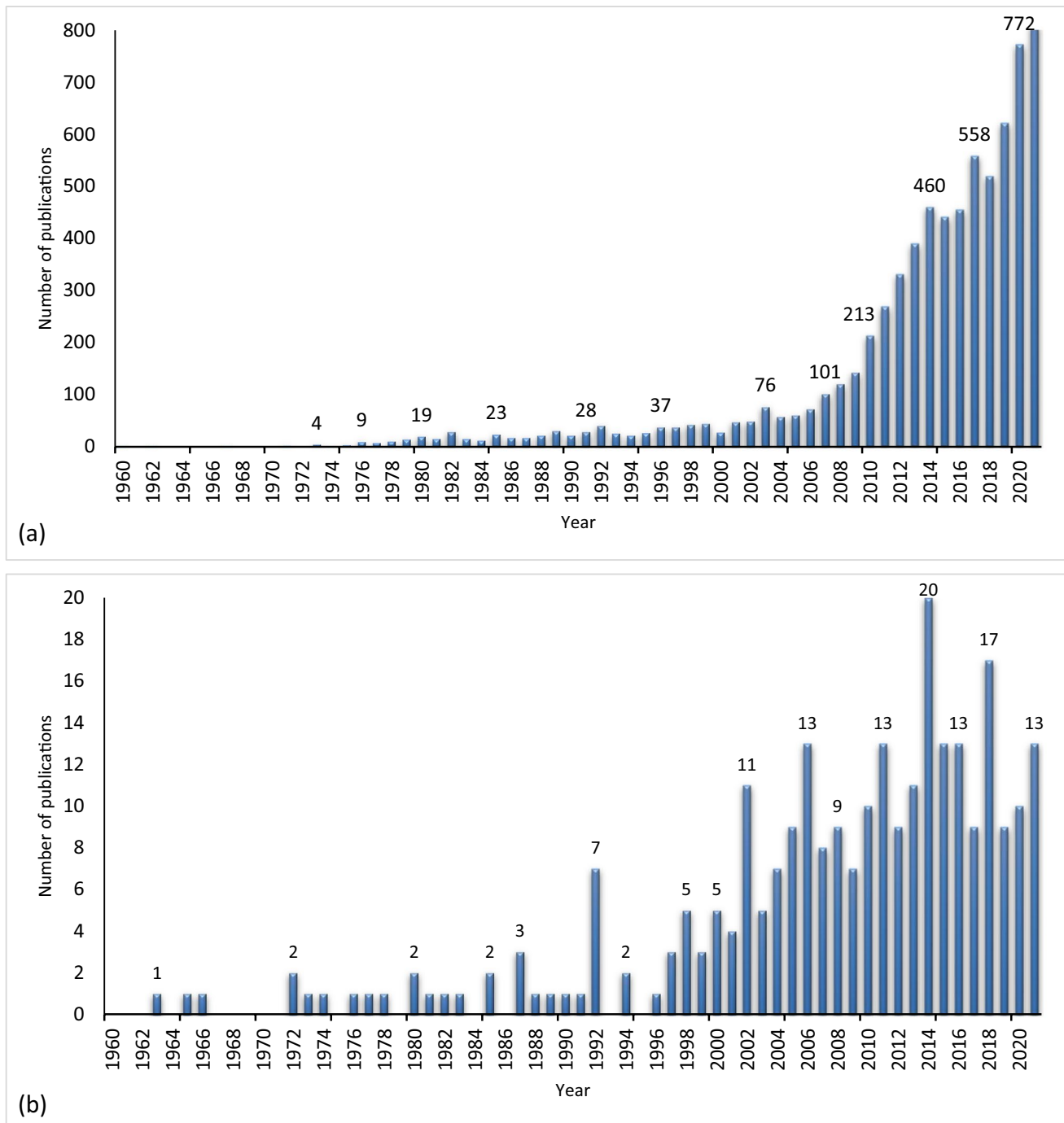
## Introduction

Preparation and analysis of whole blood and serum dried samples applied on a filter paper attracted attention of research community in the early 60-s and over the course of several years there was a rise of publication activity in human and veterinary medicine subjects. Despite the fact that Dried Blood Spot (DBS) technology today is widely used in medical diagnostics, the practical value of the technology for veterinary and biology is evidently underestimated. In fact, the use of the DBS technology for sampling, storage and dried biosamples analysis for livestock and wildlife animals disease diagnostics is not widely spread and established in veterinary practice, although, the process of

obtaining and sampling of animal dried blood samples has been described since the late 50-s and early 60-s of the last century (Adams and Hanson 1956; Karstad et al. 1957; Benson and Mickle 1964; Aldo Gaggero and Suttmöller 1965; Nobuto 1966). Over the next thirty years, this technology had not drawn much attention neither in veterinary medicine, nor in biological and ecological research. It can be noted that only since 90-s and early 2000-s, along with widespread use of the DBS technology in many research areas, the number of scientific publications concerning DBS technology began to grow (Fig. 1). However, the number of articles on DBS in veterinary and biology is still limited, and only about two hundred seventy papers in total have been published since late 50-s. During the last 10–15 years, the number of veterinary publications in DBS area did not exceed 10–20 papers per year, despite the rapid growth of general publication activity in DBS technology (Fig. 1b). Currently, DBS technology is the principal tool in neonatal screening for the rare genetic diseases detection; it is also extensively used in various medical bioanalytical studies, preclinical drug

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**Fig. 1** Annual scientific publications on DBS technology. (a) scientific publications on DBS technology (total amount, Scopus database data); (b) scientific publications on DBS technology in veterinary and biology

trials, toxicokinetic and pharmacokinetic studies, clinical pharmacology, also in forensic, doping, ecological expertise, and biobanking (Demirev 2013; Meesters and Hoeff 2013; Sharma et al. 2014; Enderle et al. 2016; Antunes et al. 2016; Lim 2018; McClendon-Weary et al. 2020). Today DBS technology is not limited to whole blood as primarily assayed biofluid and includes much wider range of utilised

dried body fluids such as urine, saliva, milk etc. The general term used for air-dried biosamples on filter paper is Dried Matrix Spots (DMS) (Jacques et al. 2022). DBS technology minimizes invasiveness of blood sampling, reduces animal mortality in preclinical studies. Moreover, dry samples are highly stable, and do not require compliance with the cold chain protocol during transportation. The difference in the

number of medical articles and veterinary/biology articles might be explained by the constant growth of DBS applications in human research and healthcare. Thus, searching scientific databases Freeman et al. (2018) identified 2018 unique analytes measured in DBS samples, among them 50% were classified as small molecule, 33% as large molecule, 15% as nucleic acid, and 2% as element. The authors also emphasized that every common analytical method applied to traditional liquid samples had been applied to DBS samples.

DBS technology definitely has distinct advantages, including simplicity of samples collection in the field, easy and space-efficient storage and delivery, applicability to small volume of biofluids, suspensions, tissues of mammals, birds, insects and other animals. Here we summarize scientific data concerning DBS technology application in veterinary medicine and biological investigations in terms of collection, shipment, storage and analysis of dried biosamples by various analytical methods.

### DBS in veterinary and biology: living objects under investigation

DBS technology has been used for samples collection and analysis in livestock, poultry, pets and in a big variety of domestic, peridomestic and wildlife mammals and birds (Table 1). Dried samples from amphibians, crustaceans, fishes, molluscs, reptiles as well insects have also been taken and investigated (Table 1). Large-scale epidemiological surveys usually included collection of dried samples from a great variety of animals. Thus, the presence of antibodies to *Yersinia pestis* was investigated in DBS samples taken from coyotes, bobcats, striped skunks, raccoons, American badgers, vulpes, rats, California voles (Wolff and Hudson 1974); gray and red foxes, gray wolves, mountain lions (Chandler et al. 2018). The investigation of wild rodents (terraced rice rats, Spix's yellow-toothed caviés, hairy-tailed bolo mice, red-nosed mice, common punarés) and synantropic rodents (rats) from an endemic area of cutaneous and visceral leishmaniasis in Brazil was described by Oliveira et al. (2005). Blood and tissues from many other vertebrates along with Australian pelicans, crested pigeons, Mallee fowls, sleepy lizards and fishes (King George Whiting, tuna), frogs, yabbies (an Australian fresh water crustacean), abalones and blue swimmer crabs were investigated by Smith and Burgoyne (2004). Domestic, synanthropic and wild mammals including dogs, donkeys, common opossums, mules, black rats, horses, fruit-eating bats, cats, wild boars, goats, red-tailed squirrels were surveyed for *Trypanosoma cruzi/Leishmania* spp coinfection (Viettri et al. 2018). Herrera et al. (2005) estimated the *Trypanosoma evansi* infection rate and epizootic status of wild and domestic mammals (equines, dogs, bats, feral pigs, peccaries, coatis), small mammals

including opossums, agoutis, spiny rats, murids from the Brazilian Pantanal region. *Trypanosoma* infection control usually included blood testing of domestic and wild animals such as horses, pigs, dogs, bovine, buffalo, zebu cattle, capybaras, coatis (Ventura et al. 2001, 2002), as well as cattle together with tsetse flies (Gillingwater et al. 2010).

### Dried samples to analyse

Application of DBS technology in veterinary and biology is not only limited to whole blood, serum and plasma (Table 1). Other blood fraction, buffy coat, applied and dried on paper, was described as a useful tool for detection of *Trypanosoma*, a blood parasite (Picozzi et al. 2002; Geysen et al. 2003; Gillingwater et al. 2010; Moti et al. 2014). Picozzi et al. (2002) noted that buffy coat preparations on filter paper matrices was the most sensitive methodology relative to the gold standard than analysis of liquid whole blood or dried whole blood for PCR determination of *T. brucei* in domestic livestock. Specific tasks in veterinary and biological research also employed a wide range of dried biosamples such as milk (Wu et al. 2008; Samsonova et al. 2014, 2017; Durel et al. 2015; Venkatesh and Gopal 2018), serosanguineous fluid from the thoracic cavity (Elmore et al. 2014), spleen and lymph node aspirates (Strauss-Ayali et al. 2004), tongue epithelial (Muthukrishnan et al. 2008; Madhanmohan et al. 2013, 2016; Biswal et al. 2016) and foot epithelium samples (Madhanmohan et al. 2016), brain tissue spots (Wacharapluesadee et al. 2003; Jefferies et al. 2007; Sakai et al. 2015; Léchenne et al. 2016; Rasolonjatovo et al. 2020), bone marrow aspirates (Cortes et al. 2004), bovine semen (Sarangi et al. 2018), haemolymph (Machado et al. 2000; Kiatpathomchai et al. 2004; Sudhakaran et al. 2009), fish body mucus and buccal cells (Lucentini et al. 2006), ray mucus (Kashiwagi et al. 2015), lavral homogenate and milt (Navaneeth Krishnan et al. 2016) and a big variety of tissue and swab samples (Table 1). Insect dried samples used for analysis included insects crash (Lall et al. 2010; Dickey et al. 2012) or suspension/homogenate (Snowden et al. 2002; Harvey 2005; Desloire et al. 2006), dry fecal spots (Russomando et al. 1996; Machado et al. 2000; Dorn et al. 2001; Brito et al. 2008; Braz et al. 2008), haemolymph (Machado et al. 2000), gut smear (Boid et al. 1999; Adams et al. 2006; Fall et al. 2012), midgut and proboscises (Gillingwater et al. 2010), crashed abdomen (Niare et al. 2017), blood meal specimens (Reeves et al. 2016) and saliva (Hall-Mendelin et al. 2010; van den Hurk et al. 2014; Flies et al. 2015; Johnson et al. 2015; Burkett-Cadena et al. 2016; Kurucz et al. 2014; 2019; Wipf et al. 2019; Birnberg et al. 2020). In some cases, insect nucleic acids were first extracted and then stored on the paper in a dried form (Owens and Szalanski 2005; Bujang et al. 2011; Miller et al. 2013). The

Table 1 DBS technology for veterinary and biology applications

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>Cattle, camels, horses</b>						
<b><i>Infectious disease diagnostics</i></b>						
<b>Bluetongue disease</b> (bluetongue virus)	Cattle, sheep	ELISA	Antibodies	Whole blood	Whatman no.4 filter paper	Afshar et al. 1987
<b>Bovine babesiosis</b> ( <i>B. bigemina</i> )	Cattle	Blocking dot ELISA, ELISA		Whole blood	Whatman filter paper	Afshar et al. 1992
	Cattle	IFA test	Antibodies	Whole blood	Whatman no.4 filter paper	Burridge et al. 1973
	Cattle				Whatman no.1 filter paper	Singh et al. 2009
	Cattle				Filter paper	Todorovic and Garcia 1978
<b>Bovine babesiosis</b> ( <i>B. bigemina</i> and <i>B. argentina</i> )					Filter paper	Young and Purnell 1980
<b>Bovine babesiosis</b> ( <i>B. divergens</i> )		ELISA		Whole blood	Filter paper (Toyo Roshi Kaisha)	El Daous et al. 2020
<b>Bovine leucosis</b> (bovine leucosis virus)	Cattle	PCR	DNA	Whole blood	Fiberglass strip	Saushkin et al. 2016a, b, 2019
<b>Bovine leucosis</b> (bovine leucosis virus), <b>bovine viral diarrhoea</b> (BVD virus), <b>infectious bovine rhinotracheitis</b> (bovine herpesvirus-1)	Cattle	ELISA, PCR	Antibodies, cDNA	Whole blood		
<b>Bovine mastitis</b> ( <i>Strep. agalactiae</i> )	Cattle	PCR	DNA	Milk	Xinhua grade 3 chr paper treated with EDTA	Wu et al. 2008
<b>Bovine mastitis</b> ( <i>S. aureus</i> , <i>Strep. agalactiae</i> and <i>M. bovis</i> )					FTA card	Durel et al. 2015
<b>Bovine respiratory disease complex</b> (bovine viral diarrhoea virus, bovine respiratory syncytial virus, bovine coronavirus, bovine herpesvirus-1)	Cattle	PCR	DNA, RNA	Diagnostic swab fluids, deep nasal swabs	FTA card	Liang et al. 2014
<b>Bovine viral diarrhoea</b> (BVD virus)	Cattle	RT-PCR	RNA	Whole blood, serum	Filtration paper, Whatman no.1 filter paper, nitrocellulose no.71002, HYBOND-M nylon	Vitček et al. 2001
<b>Brucellosis</b> ( <i>B. abortus</i> )	Cattle	ELISA	Antibodies	Whole blood	Whatman no.5 filter paper	McLean and Hilbink 1989
<b>Cystic echinococcosis</b> ( <i>Echinococcus granulosus</i> sensu lato)	Cattle, camel, sheep, goat	PCR, sequencing	DNA	Germlinal layer of cysts from liver, lung or intestines	FTA card	Boué et al. 2017

**Table 1** (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>East Coast fever (<i>Theileria parva</i>)</b>	Cattle	IFA test	Antibodies	Whole blood	Filter paper	Kimber and Burridge 1972
<b>Eastern equine encephalomyelitis (EEE virus)</b>	Horse, wild birds	Virus neutralization test	Antibodies	Whole blood, serum	Filter paper discs	Karstad et al. 1957
<b>Foot-and-mouth disease (FMD virus)</b>	Cattle	Virus neutralization test, mouse protection test	Antibodies	Whole blood, serum	Blotting paper strip	Aldo Gaggero and Sutmöller 1965
		ELISA		Serum	Fiberglass strip	Samsonova et al. 2019b
		RT-PCR	RNA	Cell culture virus, tongue epithelium	FTA card	Muthukrishnan et al. 2008
		RT-LAMP		Tongue epithelium		Madhanmohan et al. 2013
		RT-PCR, qRT-PCR, RT-LAMP		Cell culture virus, tongue and foot epithelium		Madhanmohan et al. 2016
<b>Hemoprotozoan parasites (piroplasms and trypanosomes)</b>	Camel	PCR, sequencing	DNA	Whole blood	FTA card	Sazmand et al. 2016
<b>Infectious bovine rhinotracheitis (bovine herpesvirus-1)</b>	Cattle	ELISA	Antibodies	Whole blood, serum	Whatman no.3 filter paper (strips)	de Oliveira et al. 2011
<b>Rabies (rabies virus)</b>	Cattle, horse	PCR	DNA	Bovine semen	FTA card	Saranghi et al. 2018
<b>Tropical theileriosis (<i>Theileria annulata</i>)</b>	Zebu, cross bred cattle (zebu x Friesian)	RT-PCR	RNA	Brain tissue	FTA card	Sakai et al. 2015
		PCR	DNA	Whole blood	Filter paper	Mohammed-Ahmed et al. 2018
<b>Trypanosomiasis (<i>T. brucei</i>, <i>T. congolense</i>, <i>T. vivax</i>)</b>	Cattle	IFA test	Antibodies	Whole blood	Whatman no.4 filter paper	Ashkar and Ochilo 1972
<b>Trypanosomiasis (<i>T. brucei</i>, <i>T. congolense</i>, <i>T. vivax</i>)</b>					Whatman no.1 filter paper	Platt and Adams 1976
<b>Trypanosomiasis (<i>T. evansi</i>)</b>	Water buffalo	ELISA			Whatman no.4 filter paper	Hopkins et al. 1998
						Holland et al. 2002

Table 1 (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<i>Trypanosomiasis (T. congolense and T. brucei)</i>	Cattle	PCR	DNA	Whole blood	Whatman no.41 filter paper	Katakura et al. 1997
<i>Trypanosomiasis (T. vivax, T. evanci)</i>	Cattle	PCR	DNA	Whole blood	Whatman no.4 filter paper	Ventura et al. 2001
<i>Trypanosomiasis (T. vivax, T. evanci)</i>	Cattle	PCR	DNA	Whole blood	Filter paper	Gonzales et al. 2003
<i>Trypanosomiasis (T. vivax, T. evanci)</i>	Cattle	PCR	DNA	Whole blood	Filter paper, FTA card	Gonzales et al. 2006
<i>Trypanosomiasis (T. brucei)</i>	Cattle	PCR	DNA	Whole blood, buffy coat	FTA card	Picozzi et al. 2002
<i>Trypanosomiasis (Trypanosoma species)</i>	Cattle	PCR	DNA	Whole blood	FTA card	Cox et al. 2005
<i>Trypanosomiasis (T. vivax, T. evanci)</i>	Cattle	PCR	DNA	Whole blood	FTA card	Gonzales et al. 2007
<i>Trypanosomiasis (T. congolense, T. vivax, T. brucei)</i>	Cattle	PCR	DNA	Whole blood	FTA card	Karimuribo et al. 2011
<i>Trypanosomiasis (T. brucei; Trypanosoma species)</i>	Cattle	PCR	DNA	Whole blood	FTA card	Ahmed et al. 2011, 2013
<i>Trypanosomiasis (T. vivax, T. brucei)</i>	Cattle	PCR	DNA	Whole blood	FTA card	Muhanguzi et al. 2014
<i>Trypanosomiasis (T. congolense)</i>	Cattle	PCR-RFLP	DNA	Whole blood	Whatman no.4 filter paper	Vitoulley et al. 2011
<i>Trypanosomiasis (Trypanosoma species)</i>	Cattle	PCR-RFLP	DNA	Buffy coat	Whatman no.4 filter paper	Moti et al. 2014
<i>Trypanosomiasis (Trypanosoma species)</i>	Cattle, goat	PCR-RFLP	DNA	Buffy coat	Whatman no.3 filter paper	Geysen et al. 2003
<i>Trypanosomiasis (T. congolense)</i>	Cattle, tsetse fly	PCR	DNA	Buffy coat (cattle), midgut and proboscis (tsetse fly)	FTA Elute card	Gillingwater et al. 2010
<i>Trypanosomiasis (T. vivax, T. evanci)</i>	Cattle, buffalo, sheep	PCR	DNA	Whole blood	Whatman no.4 filter paper	Dávila et al. 2003
<i>Trypanosomiasis (T. vivax, T. congolense, T. brucei)</i>	Zebu	PCR	DNA	Whole blood	FTA card	Cox et al. 2010
<i>Trypanosomiasis (T. brucei)</i>	Cattle, pig, sheep, goat	PCR	DNA	Whole blood	FTA card	de Clare Bronsvoort et al. 2010
<i>Trypanosomiasis (T. evanci)</i>	Camel	PCR	DNA	Whole blood	FTA card	Salim et al. 2011
<b>Other investigations</b>						
Pregnancy diagnosis	Cattle	ELISA	Pregnancy-associated glycoproteins (PAGs)	Whole blood	TEGO card	Sun et al. 2013
	Cattle	ELISA	Progesterone	Milk	Fiberglass strip	Samsonova et al. 2014, 2017

**Table 1** (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
Casein genotyping (A1/A2 variants of $\beta$ -casein)	Cattle	PCR	DNA	Milk, whole blood	Whatman no.3 filter paper treated with SPGA-EDTA buffer (milk) FTA card (blood)	Venkatesh and Gopal 2018
Evaluation of the nutritional status of ranging dairy cows	Zebu	ESI-MS/MS	Amino acids, acylcarnitines	Serum	Whatman 903 card	Worku et al. 2021
Atypical myopathy (inhibited $\beta$ -oxidation of fatty acids)	Horse	ESI-MS/MS	Acyl carnitines	Serum	Munktoll filter paper cards	Sander et al. 2018
Antidoping race control (anabolic steroids, $\beta$ 2-adrenoceptor agonists, corticosteroids, HIF-1 stabilizers, PPAR $\delta$ agonists, SARMs, and aromatase inhibitors)	Horse	LC-MS/MS	50 analytes	Whole blood	Whatman 903 card	Moeller and Yang 2021
<b>Small cattle</b>						
<b><i>Infectious disease diagnostics</i></b>						
<b>Toxoplasmosis (<i>T. gondii</i>)</b>	Sheep	DIG-ELISA	Antibodies	Whole blood	Munktoll 1300 filter paper	Uggla and Nilsson 1987
	Sheep, goat	ELISA, IFA test		Serum	Whatmann no.4 filter paper	Al-Kappany et al. 2018
<b>Trypanosomosis (<i>Trypanosoma</i> spp.)</b>	Goat	PCR	DNA	Whole blood	Whatman no.4 filter paper	de Almeida et al. 1997, 1998a, b
<b>Trypanosomosis (<i>T. congolense</i>, <i>T. vivax</i>, <i>T. brucei</i>)</b>	Goat	PCR	DNA	Whole blood	Whatman no.4 filter paper	Almadu et al. 2002
<b>Viral arthritis-encephalitis (CAEV virus), toxoplasmosis (<i>T. gondii</i>)</b>	Goat	ELISA, latex agglutination	Antibodies	Whole blood, serum	Fiberglass strip	Saushkin et al. 2018
<b>Other investigations</b>						
Molecular detection and genotyping of Peste des petits ruminants virus	Goat	RT-PCR; sequencing	RNA	Whole blood, nasal swabs	Whatman 3MM filter paper	Bhuiyan et al. 2014
<b>Swine</b>						
<b><i>Infectious disease diagnostics</i></b>						
<b>African swine fever (ASF virus)</b>	Pig	PCR, sequencing PCR, ELISA	DNA DNA, antibodies	Whole blood	FTA card Whatman 3MM filter paper (stripes)	Utenthal et al. 2013 Randriamparany et al. 2014

Table 1 (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>Aujeszky's disease (AD virus)</b>	Pig	ELISA	Antibodies	Whole blood	Whatman no.1 filter paper	Banks 1985
<b>Foot-and-mouth disease (FMD virus)</b>	Pig	Virus neutralization test, ELISA	Antibodies	Whole blood, serum	Filter paper	Motha et al. 1987
<b>Porcine reproductive and respiratory syndrome (PRRS virus)</b>	Pig	ELISA	Antibodies	Whole blood	Whatman no.113 filter paper	Armstrong et al. 1991
		ELISA, RT-PCR	Antibodies, RNA		Filter paper	Hutet et al. 2003
		RT-PCR	RNA	Whole blood	TEGO Card	Yoon et al. 2010
					Filter paper	Spagnuolo-Weaver et al. 1998
<b>Swine influenza (swine influenza virus)</b>	Pig	RT-PCR	RNA	Whole blood, serum, organs (lung, tonsil, and superficial inguinal lymph nodes), oral fluid	FTA card	Inoue et al. 2007
<b>Swine vesicular disease (SVD virus)</b>	Pig	ELISA	Antibodies	Allantoic fluid, nasal swabs, lung tissue	FTA card	Linhares et al. 2012
<b>Toxoplasmosis (<i>T. gondii</i>)</b>	Pig	HA test	Antibodies	Whole blood	Filter and blotting paper	Hamblin and Hedger 1982
<b>Vesicular stomatitis (VS virus)</b>	Pig	Virus neutralization test	Antibodies	Serum	Nobuto filter paper strip	Nobuto 1966
<b>Other investigations</b>					Blotting paper	Adams and Hanson 1956
<b>Pregnancy diagnosis</b>	Pig	RIA	Progesterone	Whole blood	Nobuto filter paper strip	Lin et al. 1988
<b>Genotyping and phylogenetic analysis of African swine fever virus, Peste des petits ruminants virus</b>	Pig, African dwarf goat	PCR, sequencing	DNA; RNA	Whole blood	Schleicher and Schuell 2992 filter paper	Chadio et al. 2002
<b>Extraction of DNA for genomic analysis</b>	Pig	WGA, STR-PCR, SNP	DNA	Whole blood	Whatman 3MM filter paper, FTA card	Michaud et al. 2007
<b>Determining of optimal dietary intake of nutrients</b>	Pig	Analysis was done by Lipid Technologies, LLC (USA)	Fatty acids	Whole blood	FTA card	Fowler et al. 2012
<b>Poultry</b>						
<b>Infectious disease diagnostics</b>						
						Wood et al. 2021a



**Table 1** (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>Avian infectious bronchitis</b> (infectious bronchitis virus)	Chicken	ELISA	Antibodies	Whole blood	Whatman no. 1 filter paper (strips)	Lana et al. 1983
<b>Avian influenza</b> (avian influenza virus)	Chicken, wild birds	RT-PCR, RFRP, sequencing	RNA	Tracheal swabs	FTA card	Moscoso et al. 2005
	Chicken	RT-qPCR RT-PCR	RNA	Swab samples Cloacal and oropharyngeal swabs, lungs, spleen, kidneys, and brain	FTA card	Abdelwhab et al. 2011 Jóźwiak et al. 2016
<b>Avian influenza</b> (avian influenza virus), <b>Newcastle disease</b> (Newcastle disease virus)	Chicken	HI test	Antibodies	Whole blood	Filter paper	Brugh and Beard 1980
	Poultry (chicken, turkey, guinea fowl, duck, goose)	RT-PCR	RNA	Oropharyngeal swabs	FTA card	Shekaili et al. 2015
<b>Avian metapneumovirus infection</b> (avian metapneumovirus)	Chicken	RT-PCR	RNA	Turbinate, trachea and lung	FTA card	Awad et al. 2014
<b>Fowl adenovirus I</b> detection and genotyping	Chicken	PCR	DNA	Liver	FTA card	Moscoso et al. 2007
<b>Fowl cholera</b> ( <i>P. multocida</i> )	Chicken	ELISA	Antibodies	Whole blood	Schleicher & Schuell no.740-E filter paper (strips)	Avakian and Dick 1985
<b>Fowl typhoid and paratyphoid</b>	Chicken	ELISA	Antibodies	Whole blood	Filter paper	Minga et al. 1992
	Chicken	ELISA	Antibodies	Whole blood	Filter paper	Minga and Wray 1992
<b>Hepatitis B</b> (duck hepatitis virus)	Duck	PCR	DNA	Serum	Whatman no.1 filter paper, nitrocellulose no.71002, HYBOND-M nylon	Wang et al. 2002
<b>Infectious bursal disease</b> (infectious bursal disease virus)	Chicken	Agar gel precipitation test	Antibodies	Whole blood	Whatman no.1 filter paper (strips)	Roy et al. 1994
		QAGP test		Serum	Whatman no.1 filter paper	Thangavelu et al. 2000
		ELISA	RNA	Bursa	FTA card	Ahmed et al. 2012
		RT-PCR				Moscoso et al. 2006 Purvis et al. 2006 Maw et al. 2006
					Chromatography paper, filter paper, stationery copy paper, FTA card	

Table 1 (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>Infectious bursal disease</b> (infectious bursal disease virus), <b>avian infectious bronchitis</b> (infectious bronchitis virus), <b>micoplasmosis</b> ( <i>M. gallisepticum</i> and <i>M. synoviae</i> )	Chicken	ELISA	Antibodies	Whole blood, serum	Fiberglass strip	Samsonova et al. 2019a
<b>Marek's disease</b> (Marek's disease virus)	Chicken	PCR	DNA	Whole blood, solid tumors	FTA card	Cortes et al. 2009
<b>Micoplasmosis</b> ( <i>M. gallisepticum</i> and <i>M. synoviae</i> )	Chicken	PCR, RFRP, sequencing	DNA	Imprints of tumors Tracheal swabs	FTA card	Gimeno et al. 2014 Moscoso et al. 2004
<b>Newcastle disease</b> (Newcastle disease virus)	Chicken	HI test	Antibodies	Whole blood	Nobuto filter paper strip Whatman no.1 filter paper	Beard and Brugh 1977 Roy et al. 1997
		RT-PCR	RNA	Trachea, lung, caecal tonsil and cloacal faeces	Whatman no.1 filter paper (strips) FTA card	Roy et al. 1992 Perozo et al. 2006
<b>Western equine encephalomyelitis</b> (WEE virus), <b>St. Louis encephalitis</b> (SLE virus)	Chicken	ELISA	Antibodies	Whole blood	Whatmann no.1 filter paper (strips)	Narayanan et al. 2010 Reisen et al. 1994
<b>Other investigations</b>						
Antibiotic detection	Chicken	LC	Enrofloxacin, ciprofloxacin	Whole blood	Filter paper	Posyniak et al. 2002
Determination of avian sexing	Chicken	PCR	DNA	Whole blood	FTA Elute card, FTA card, Whatman 903 filter paper, office paper	Suriyaphol et al. 2014
Genotyping of infectious bronchitis virus	Chicken	RT-PCR, sequencing	RNA	Tissues of trachea, lung, kidney and caecal tonsil Tissues and swab samples Tissues of trachea, pharynx, caecal tonsil, kidney and turbinate	FTA card	Ganapathy et al. 2015 Manswr et al. 2018 Ball et al. 2016

**Table 1** (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>Pets</b>						
<b>Infectious disease diagnostics</b>						
<b>Canine babesiosis</b> ( <i>B. gibsoni</i> )	Dog	PCR	DNA	Whole blood	Whatman 3MM filter paper	Tani et al. 2008
<b>Canine distemper</b> (CD virus), <b>infectious canine hepatitis</b> (canine adenovirus 1), <b>leptospirosis</b> ( <i>Leptospira</i> spp.)	Dog	Virus neutralization test	Antibodies	Whole blood	Borosilicate filter paper no. 934-AH	Benson and Mickle 1964
<b>Canine visceral leishmaniasis</b> ( <i>L. donovani</i> )	Dog	IFA test	Antibodies	Whole blood	Filter paper	Evans et al. 1990 Cabrera et al. 1999 da Silva et al. 2000 Figueiredo et al. 2010a, b
		DAT			Whatman no.4 filter paper	Kalayou et al. 2011
		ICT	Plasma		HemaSpot device	Rosypal et al. 2014
		PCR	DNA	Bone marrow aspirates, whole blood	Whatman no.3 filter paper	Cortes et al. 2004
				Spleen or lymph node aspirates, whole blood	Filter paper	Strauss-Ayali et al. 2004
<b>Dirofilariasis</b> ( <i>D. repens</i> )	Dog	qPCR	DNA	Whole blood	Whatman FTA Elute card	Duscher et al. 2009
<b>Toxoplasmosis</b> ( <i>T. gondii</i> )	Cat	Latex agglutination test	Antibodies	Whole blood	Quantitative filter paper strips (Toyo Roshi Kaisha)	Nogami et al. 1992
		MAT			Whatman 903 card	Bolais et al. 2017
<b>Trypanosomiasis</b> ( <i>T. brucei gambiense</i> )	Dog	PCR	DNA	Whole blood	FTA card	Umeakuana et al. 2019
<b>Piroplasmosis</b> (piroplasm spp.)	Dog	PCR-RFLP	DNA	Whole blood	FTA classic card (cut into strips) and IsoCode stix	Jefferies et al. 2007
<b>Rabies</b> (rabies virus)	Dog	RT-PCR, NASBA	RNA	Brain tissue	Schleicher and Schuell no. 903 filter paper	Wacharaphuesadee et al. 2003
		RT-qPCR			FTA card	Léchenne et al. 2016
<b>Other investigations</b>						
Genotyping of dog leukocyte antigen	Dog, wolf	PCR, sequencing	DNA	Whole blood	Whatman FTA Elute card	Kennedy et al. 2008
Control of immune-mediated and allergic disorders	Cat	HPLC-ESI-MS/MS	Cyclosporin A	Whole blood	Whatman 903 card	Mohamed et al. 2012

Table 1 (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
Lysosomal storage disease	Dog, cat	Fluorescent method	Lysosomal enzymes activity	Whole blood	Munktel TFN 06–079 card	Sewell et al. 2012
Determination of the prevalence of vector-borne pathogens ( <i>Rickettsia</i> spp., Anaplasmataceae, <i>Bartonella</i> spp. and <i>Babesia</i> spp)	Dog	PCR	DNA	Whole blood	FTA card	Probst et al. 2015
Telomere length assessment	Dog	qPCR	DNA	Whole blood	FTA card	Dutra et al. 2020
<b>Wild and domestic animals (mammals and birds)</b>						
<b>Infectious disease diagnostics and screening</b>						
<b>Aleutian disease</b> (Aleutian disease virus)	Mink	ELISA	Antibodies	Whole blood	12-spot DBS card (comb)	Dam-Tuxen et al. 2014; Knuutila et al. 2014
<b>Avian influenza</b> (avian influenza virus)	Mallard Mallard	ELISA RT-PCR, sequencing	Antibodies RNA	Whole blood Allantoic fluid, cloacal swabs	Munktel TFN filter paper Nobuto filter paper strips FTA card	Andersson et al. 2015, 2016, 2017 Dusek et al. 2011 Kraus et al. 2009, 2011
<b>Bluetongue disease</b> (bluetongue virus), <b>hemorrhagic disease</b> (hemorrhagic disease virus)	Waterfowl	RT-PCR	Antibodies	Cloacal and oropharyngeal swabs		Keeler et al. 2012
<b>Sero-prevalence of bluetongue disease</b> (bluetongue virus), <b>hemorrhagic disease</b> (hemorrhagic disease virus)	White-tailed deer Mule deer	Virus neutralisation test, AGID Virus neutralization test	Antibodies	Whole blood	Schleicher & Schuell no. 740E filter paper (stripes)	Stallknecht and Davidson 1992 Dubay et al. 2006
<b>Bovine tuberculosis</b> ( <i>M. bovis</i> )	Wild boar	ELISA	Antibodies	Whole blood	Whatman 903 card, FTA card	Santos et al. 2018
<b>Sero-prevalence of brucellosis</b> ( <i>B. abortus</i> )	Arctic caribou	ELISA	Antibodies	Whole blood	Nobuto filter paper strips	Curry et al. 2011
<b>Brucellosis</b> ( <i>B. abortus</i> )	Rocky Mountain elk	PFIA	Antibodies	Whole blood		Jennings-Gaines et al. 2021
<b>Assessment of exposure to <i>Brucella abortus</i>, <i>Neospora caninum</i>, West Nile virus, herpes virus, bovine parainfluenza virus, bovine respiratory syncytial virus, bovine viral diarrhoea virus</b>	Caribou, reindeer	ELISA; virus neutralization test	Antibodies	Whole blood	Nobuto filter paper strips	Curry et al. 2014b

**Table 1** (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
Sero-prevalence of <b>canine distemper virus, canine parvovirus type 2, and canine adenovirus type 1</b>	Wolverines	IFA	Antibodies	Whole blood	Nobuto paper strip	Dalerum et al. 2005
<b>Canine distemper</b> (canine distemper virus), <b>canine parvovirus disease</b> (canine parvovirus)	Coyote, raccoon	Virus neutralization test; HI test	Antibodies	Whole blood	Nobuto filter paper strips	Kamps et al. 2015
Sero-prevalence of <b>cat scratch disease – bartonellosis</b> ( <i>B. henselae</i> )	Puma, bobcat Mountain lion, bobcat	IFA test	Antibodies	Whole blood	Nobuto filter paper strips	Chomel et al. 2004 Yamamoto et al. 1998
Sero-prevalence of <b><i>Bartonella visonii</i> subsp. <i>berkhoffii</i></b>	Coyote	ELISA				Chang et al. 1999
<b>Cytauxzoonosis</b> ( <i>C. felis</i> )	Bobcat	PCR	DNA	Whole blood	Nobuto filter paper strips	Birkenheuer et al. 2008
<b>Canine heartworm disease – dirofilariasis</b> ( <i>D. immitis</i> )	Coyote	ELISA	Antibodies	Whole blood	Nobuto filter paper strips	Sacks et al. 2002
Investigation of <b><i>Ehrlichia</i> spp.</b> occurrence	Coyote	PCR	DNA	Whole blood	Nobuto filter paper strips	Pusterla et al. 2000
Sero-prevalence of <b><i>Ehrlichia chaffeensis</i></b>	White-tailed deer	IFA	Antibodies			Mueller-Anneling et al. 2000
Sero-prevalence of <b>hepatitis E</b> (hepatitis E virus)	Sika deer	ELISA	Antibodies	Whole blood	Whatman filter paper discs	Yu et al. 2007
Sero-prevalence of <b>louping-ill virus</b>	Willow ptarmigan	HI test	Antibodies	Whole blood	Nobuto filter paper strips	Yirehus et al. 2021
<b>Lyme disease</b> ( <i>B. burgdorferi</i> ), <b>anaplasmosis</b> ( <i>A. phagocytophilum</i> ), <b>ehrlichiosis</b> ( <i>E. canis</i> ), <b>canine heartworm disease disease – dirofilariasis</b> ( <i>D. immitis</i> )	Grey wolf	ELISA (SNAP 4Dx test)	Antibodies	Serum	Nobuto filter paper strips	Jara et al. 2015
Assessment of exposure to <b><i>Neospora caninum</i></b> , <b>West Nile virus</b> , <b>herpes virus</b> , <b>bovine parainfluenza virus</b> , <b>bovine respiratory syncytial virus</b> , <b>bovine viral diarrhoea virus</b>	Reindeer	ELISA; virus neutralization test	Antibodies	Whole blood	Nobuto filter paper strips	Curry et al. 2014a
<b>Pigeon circovirus infection</b> (pigeon circovirus)	Pigeon	PCR	DNA	Whole blood	Filter paper	Hattermann et al. 2002

Table 1 (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>Plague (<i>Y. pestis</i>)</b>	Small rodents, carnivores	HA test	Antibodies	Whole blood, serum	Nobuto filter paper strips	Wolff and Hudson 1974
	Coyote, raccoon, canids, felids	HI test; a bead-based flow cytometric serodiagnostic assay (Luminex)		Whole blood		Chandler et al. 2018
Assessment of <b>plague (<i>Y. pestis</i>)</b> exposure	Coyote	ICT				Abbott et al. 2014
<b>Rabies</b> (rabies virus)	Red fox, raccoon dog	ELISA	Antibodies	Whole blood	Trans-blot filter paper, BioRad	Wasniewski et al. 2014
Assessment of prevalence of <b>reticuloendotheliosis virus</b>	Wild animals	RT-hn-PCR	RNA	Brain tissue	Whatman 903 filter paper	Rasolonjatovo et al. 2020
<b>Sarcocystosis</b> ( <i>S. falcatula</i> )	Wild turkey	PCR	DNA	Whole blood	Whatman 3MM filter paper	Stewart et al. 2019
Sero-prevalence of <b>Sendai virus, mouse hepatitis virus</b>	Thick-billed parrot	PCR	DNA	Whole blood	FTA card	Rivas et al. 2021
	Mice	ELISA	Antibodies	Whole blood	Filter paper discs (Advance Toyo)	Katakura et al. 1992
Sero-prevalence of <b>toxoplasmosis</b> ( <i>T. gondii</i> ), <b>sarcocystosis</b> ( <i>S. neurona</i> )	Beaver	MAT	Antibodies	Whole blood	Filter paper	Jordan et al. 2005
<b>Toxoplasmosis</b> ( <i>T. gondii</i> )	Zoo animals (mammals and birds)	Indirect HA test	Antibodies	Whole blood	Schleicher & Schuell no.589 filter paper	Ippen et al. 1981
Sero-prevalence of <b><i>Toxoplasma gondii</i></b>	Bobcat	MAT			Nobuto filter paper strips	Mucker et al. 2006
	Domestic and peridomestic rodents				Guthrie card	Mercier et al. 2013
	Ungulates (tapir, peccary, brocket deer)				Whatman 903 card, FTA card, cellulose filters grade 2 and 3	Aston et al. 2014
	Arctic-nesting geese	IFA test, direct agglutination test		Serosanguineous fluid from the thoracic cavity	Nobuto filter paper strips	Elmore et al. 2014
	Wolverines	MAT, ELISA, IFA test		Whole blood		Sharma et al. 2019

**Table 1** (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>Trypanosomiasis (<i>T. evansi</i>)</b>	Wild and domestic animals (horse, pig, dog, bovine, buffalo, capybara, coatis)	PCR	DNA	Whole blood	Whatman no.4 filter paper	Ventura et al. 2002
	Wild and domestic mammals (equines, dog, bat, feral pig, peccary, coatis and small mammals)				Filter paper confetti	Herrera et al. 2005
<b>Trypanosomiasis (<i>T. cruzi</i>)</b>	Non-human primates				FTA card	Aysanoa et al. 2017
	Bat				Whatman 3MM filter paper	Villena et al. 2018
	Canine, skunk				Nobuto filter paper strips	Gulas-Wroblewski et al. 2021
<b>Trypanosomiasis: diagnosis and genetic analysis</b>	Rat, native rodents				Filter paper	Ortiz et al. 2018
<b>Trypanosomiasis and leishmaniasis coinfection (<i>T. cruzi/Leishmania</i> spp)</b>	Domestic, sinantropic and wild mammals	PCR	DNA	Whole blood	Filter paper	Vietri et al. 2018
<b>Tularemia (<i>F. tularensis</i>)</b>	Mice, ticks	PCR-EIA	DNA	Whole blood, liver and spleen smears (mice), tick extracts	FTA card	Higgins et al. 2000
<b>Visceral leishmaniasis (<i>Leishmania</i> spp.)</b>	Small rodents	PCR-hybridization technique	DNA	Whole blood	FTA card	Oliveira et al. 2005
<b>Sero-prevalence of West Nile virus infection (West Nile virus)</b>	Red-winged blackbird	ELISA	Antibodies	Whole blood	Nobuto filter paper strips	Sullivan et al. 2006
	Ruffed grouse	PRNT				Nemeth et al. 2017; 2021
<b>West Nile virus surveillance</b>	Wild birds (corvid, passerine, raptor, waterfowl)	RT-PCR	RNA	Oral swabs	FTA card, RNASound card	Foss et al. 2016
<b>Genetic studies</b>						
Isolation and characterization of DNA microsatellite markers	Kirtland's warbler	PCR, sequencing	DNA	Whole blood	FTA card	King et al. 2005
Phylogenetic analysis of simian T lymphotropic virus	Monkeys	PCR-based genome walking approach, sequencing	DNA	Whole blood	Whatman filter paper	Sintasath et al. 2009a, b

Table 1 (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
Population genetic and phylogenetic analysis	Indigenous chickens, Ceylon junglefowl	PCR, sequencing	DNA	Whole blood	FTA card	Silva et al. 2009
Population genetic studies	Coyote	Microsatellite genotyping	DNA	Whole blood	Nobuto filter paper strips	Sacks et al. 2004
	Mallard	SNP genotyping			FTA card	Kraus et al. 2013
	Barn swallow	PCR			Whatman filter paper	Guerrini et al. 2014
	Lemur	Microsatellite genotyping			FTA card	Nunziata et al. 2016
Microsatellite genotyping	Addax	PCR	DNA	Whole blood	FTA card	Heim et al. 2012
Verification of taxonomic identifications in ecological surveys	Opossum, bat, rodents	DNA barcoding	DNA	Liver, heart, other tissues	FTA CloneSaver card	Borisenko et al. 2008
Molecular characterization of piropiasm species ( <i>Babesia</i> and <i>Cytauxzoon</i> )	Meerkat	PCR, sequencing	DNA	Whole blood	FTA elute card	LeClaire et al. 2015
<b>Ecological investigations</b>						
Exposure to brevetoxin-producing red tides	Bottlenose dolphin	ELISA	Brevetoxin	Whole blood	Schleicher and Schuell grade 903 filter paper	Maucher et al. 2007
Exposure to multiple algal toxins			Brevetoxin and domoic acid			Twiner et al. 2011
Exposure to environmental contaminants	Wild birds	ICP-MS, HRGC-HRMS, LC + ESI-MS/MS	Heavy metals, organochlorine pesticides, toxic organic substances	Whole blood	Schleicher and Schuell no. 903 filter paper	Shlosberg et al. 2011, 2012
	Wild birds	GC-ECD	Toxic chlorinated hydrocarbons		Whatman 903 card	Lehner et al. 2018
	Bald eagle, African penguin	GC-MS/MS	Organochlorine pesticides and polychlorinated biphenyls			Lehner et al. 2020
	Florida manatee	UHPLC-MS/MS	Per- and polyfluoroalkyl substances			Griffin et al. 2021
Assessment of toxic heavy metals exposure	Eagle, cat, crocodile	ICP-MS	Toxic heavy metals	Whole blood	Whatman 903 card	Lehner et al. 2013



**Table 1** (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
Monitoring mercury concentration in wildlife population	Marine mammals (bottlenose dolphin, harbor seal)	AAS	Mercury	Whole blood	Nobuto filter paper strips	Hansen et al. 2014
Risk assessment of mercury concentration	Pinnipeds (northern elephant seal, harbor seal, California sea lion)					McHuron et al. 2019
Assessment of mercury exposure	Wild birds (zebra finch, American golden-clover)				Whatman 903 card	Perkins and Basu 2018
<b>Other investigations</b>						
Assessment of feeding ecology or diet studies	Bottlenose dolphin, muskox, wild moose	EA-IRMS	Carbon (C) and nitrogen (N) stable isotopes	Whole blood	Nobuto filter paper strip	O'Hara et al. 2018
Assessment of the vitamin D3 status (metabolic bone disease, MBD) of indoor housed birds	Tropical birds Cormorant	Analysis (LC-MS/MS) was done by City Assays (UK)	25-Hydroxyvitamin D3	Whole blood	Whatman 903 filter paper	Drake et al. 2017 Jaffe et al. 2019
The evaluation of vitamin D status	Chimpanzee Hoffmann's two-toed sloth	LC-MS/MS	25-hydroxyvitamin D	Whole blood	Whatman 903 filter paper	Moititié et al. 2020 Higgins et al. 2020
Evaluating of circulating fatty acid composition	African savanna elephant Southern white rhinoceros	Analysis (GC) was done by Lipid Technologies, LLC (USA)	Fatty acids	Whole blood	PerkinElmer spot saver card	Wood et al. 2021b Wood et al. 2021c
Cholinesterase inhibitors (e.g. organophosphate and carbamate insecticides)	Wild birds	Spectrophotometric method	Cholinesterase activity	Whole blood	Whatman no.4 filter paper	Trudeau et al. 2007
Collecting and archiving of DNA species	Vertebrates (mammals, birds, fish, reptiles), invertebrates	PCR, RFLP	DNA	Whole blood, tissues	FTA card	Smith and Burgoyne 2004
DNA extraction optimisation for genetic studies	Elk, domestic dogs, Rocky Mountain bighorn sheep, mule deer	PCR	DNA	Whole blood	Nobuto filter paper strip, FTA card, FTA Elute card	Love Stowell et al. 2018

Table 1 (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
Determination of avian sexing	Great Grey Shrike	PCR	DNA	Whole blood	FTA card	Gutiérrez-Corchero et al. 2002
	Seabirds				Common filter paper	Quintana et al. 2009
	Fighting cock, sun conure				Whatman grade 1 filter paper	Asawakarn et al. 2018
Stork hybrids diagnostics	Stork	PCR	DNA	Whole blood	FTA card	Yee et al. 2013
<b>Amphibians, crustaceans, fishes, molluscs, reptiles</b>						
<b><i>Infectious disease diagnostics</i></b>						
<b>Ranavirus infection</b> (ranavirus)	Green frog	PCR	DNA	Whole blood	FTA card	Forzán and Wood 2013
<b>Viral nervous necrosis</b> (betanodavirus)	Asian seabass	RT-PCR	RNA	Tissue homogenate, cell culture supernatant, gonadal fluid-milt and seawater spiked with betanodavirus	FTA card	Navaneeth Krishnan et al. 2016
<b>White spot disease</b> (white spot syndrome virus)	Shrimp	PCR	DNA	Haemolymph	FTA card	Sudhakaran et al. 2009
<b>Yellow head virus infection</b> (YH virus)	Shrimp	RT-PCR	RNA	Haemolymph	ISOCODE filter paper, Schleicher & Schuel	Kiatpathomchai et al. 2004
<b>Other investigations</b>						
Microsatellite and RFLP genotyping	Northern pike, brown trout	PCR–RFLP	DNA	Body mucus, buccal cells smears	FTA card	Lucentini et al. 2006
Microsatellite genotyping and mitochondrial sequencing	Abalon	PCR, sequencing	DNA	Squash tissue samples (foot, epipodial tentacles, whole juveniles)	FTA card	Carr and Appleyard 2008
Population genetic studies	Manta ray	PCR	DNA	Mucus	FTA Elute card	Kashiwagi et al. 2015
Assessment of the vitamin D3 status (nutritional metabolic bone disease, NMBD) in captive animals	Oriental fire-bellied toad	Analysis (LC–MS/MS) was done by City Assays (UK)	25-Hydroxyvitamin D3	Whole blood	Test blotting strips	Michaels et al. 2015
Assessment of wild-type diet composition and examination of fatty acid status for wild and managed care animals	Turtle	Analysis (GC) was done by Lipid Technologies, LLC (USA)	Fatty acids	Whole blood	PerkinElmer 226 spot saver RUO card	Dass et al. 2020; Koutsos et al. 2021
Part of an annual continuing health assessment	San Cristóbal Galápagos tortoise					Dass et al. 2021
Assessing of mercury exposure and dietary carbon sources	Arctic char	AAS, GC–C-IRMS	Mercury, stable carbon isotope fingerprinting of essential amino acids	Whole blood	Whatman 903 filter paper	Barst et al. 2020

**Table 1** (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
Determining of trophic position	Arctic char	GC-C-IRMS	Stable nitrogen isotope ratios of amino acids	Whole blood	Whatman 903 filter paper	Barst et al. 2021
<b>Insects</b>						
<b><i>Detection/identification of infectious agent</i></b>						
<i>Thelohania solenopsae</i> (microspora)	Fire ant ( <i>Solenopsis invicta</i> )	PCR	DNA	Ant homogenate	FTA card	Snowden et al. 2002
<i>Trypanosoma cruzi</i>	Triatomine ( <i>Triatominae infestans</i> )	PCR	DNA	Dry fecal spots	Whatman no.1 filter paper Filter paper 1064 FRAMA Filter paper	Russomando et al. 1996 Braz et al. 2008 Dorn et al. 2001
<i>Trypanosoma cruzi</i> , <i>Trypanosoma rangeli</i>	Triatomine ( <i>Rhodnius prolixus</i> ) Triatomine ( <i>Dipetalogaster maximus</i> )			Feces and hemolymph	Filter paper	Machado et al. 2000
<i>Trypanosoma</i> identification	Tsetse fly			Midgut	FTA card	Adams et al. 2006
<i>Trypanosoma evansi</i> , blood meal identification	Haematophagous diptera ( <i>Stomoxys calcitrans</i> )	ELISA, PCR	Antibodies, DNA	Gut smear	Whatman no.1 filter paper	Boid et al. 1999
Alphaviruses and flaviviruses	Mosquito	RT-PCR, sequencing	RNA	Mosquito saliva	Honey-soaked FTA card	Wipf et al. 2019 Birnberg et al. 2020
Alphameasovirus, quaranjavirus (Wuhan mosquito virus), unclassified Bunyavirales		NGS metagenomics analysis, RT-PCR				
Kunjin virus and Alfuy virus		RT-PCR				Kurucz et al. 2019 Kurucz et al. 2014 Flies et al. 2015
Murray Valley encephalitis virus						Johnson et al. 2015
Ross River virus, Barmah Forest virus, Stratford virus						
Ross River virus, Barmah Forest virus, Murray Valley encephalitis virus, West Nile virus (Kunjin subtype)						
West Nile virus, eastern equine encephalitis virus						
West Nile virus Kunjin subtype, Ross River virus, Barmah Forest virus		RT-PCR, sequencing				Burkett-Cadena et al. 2016 van den Hurk et al. 2014
West Nile virus, Ross River virus, Chikungunya virus, Barmah Forest viruses		RT-PCR			Honey-soaked FTA card, filter paper	Hall-Mendelin et al. 2010

Table 1 (continued)

*Infection/causative agent/aim of the investigation	Living object	**Method	Analyte	Dried sample	Sample carrier	Reference
<b>Other investigations</b>						
Insects identification (forensic applications)	Calliphorids	PCR	DNA	Insect homogenate	FTA card	Harvey 2005
Sample preservation and storage (insects and pathogen DNA)	House fly	PCR	DNA	Nucleic acid extract	Qualitative filter paper	Owens and Szalanski 2005
Specimen collection and storage	Aphid specimens	PCR, sequencing	DNA	The purified DNA or the solution of the cell lysis buffer	Filter Paper Qualitative P5 (Thermo Fisher Scientific), FTA card	Miller et al. 2013
DNA extraction optimisation	Chicken mite ( <i>Dermanyssus gallinae</i> ) Whitefly Triatomine	PCR LSSP-PCR	DNA DNA	Mite suspension Smashed insects Fecal spots	FTA card FTA plant card FTA card	Desloire et al. 2006 Dickey et al. 2012 Brito et al. 2008
Genotyping of <i>T. cruzi</i> (genetic polymorphism)	Tsetse fly	AFLP technique	DNA	Insect crash	FTA card	Lall et al. 2010
Amplification of endo- $\beta$ -1,4-glucanase gene	Termit	PCR	DNA	Nucleic acid extract	FTA plant card	Bujang et al. 2011
Taxonomic identification of vector blood meal	Mosquito	PCR	DNA	Blood meal specimens	FTA card	Reeves et al. 2016
Blood meal identification		ELISA	Antibodies	Gut	Whatman filter paper	Fall et al. 2012
Blood meal identification (fed on human, sheep, rat, rabbit, dog and chicken blood)		MALDI-TOF MS	MS spectra	Crushed abdomen	Filter paper	Niare et al. 2017

\*AD virus—Aujeszky disease virus, ASF virus—African swine fever virus, BVD virus—bovine viral diarrhoea virus, CAEV virus—caprine arthritis-encephalitis virus, CD virus—canine distemper virus, EEE virus—Eastern equine encephalomyelitis virus, FMD virus—foot-and-mouth disease virus, HIF-1—hypoxia inducible factor-1, PPAR—peroxisome proliferator-activated receptor, PRRS virus—porcine reproductive and respiratory syndrome virus, SARM—selective androgen receptor modulators, SLE virus—St. Louis encephalitis virus, SVD virus—swine vesicular disease virus, VS virus—vesicular stomatitis virus, WVE virus—Western equine encephalomyelitis virus, YH virus—yellow head virus

\*\*AAS—atomic absorption spectrometry, AFLP—amplified fragment length polymorphism, AGID—agar gel immunodiffusion test, DAT—direct agglutination test, DIG-ELISA—the diffusion-in-gel enzyme-linked immunosorbent assay, EA-IRMS—elemental analysis—ratio mass spectrometry, ESI-MS/MS—electrospray tandem mass spectrometry, GC-C-IRMS—gas chromatography-combustion-isotope ratio mass spectrometry, GC-ECD—gas chromatography with electron capture detection, GC-MS/MS—gas chromatography tandem quadrupole mass spectrometry, HA—haemagglutination, HI—haemagglutination inhibition, HPLC-ESI-MS/MS—high pressure liquid chromatography hyphenated to positive electrospray tandem mass spectrometry, HRGC-HRMS—high resolution gas chromatography-mass spectrometry, ICP-MS—inductively coupled plasma mass spectrometry, ICT—immunochromatographic test (lateral flow immunoassay), IFA—indirect fluorescent antibody, KDRT—Kalazar detect rapid test, LC+ESI-MS/MS—liquid chromatography electrospray ionization mass spectrometry, LC-MS/MS—liquid chromatography-tandem mass spectrometry, LSSP-PCR—the low-stringency single specific primer—polymerase chain reaction, MALDI-TOF MS—matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MAT—modified agglutination test, MS/MS—tandem mass spectrometry, NASBA—nucleic acid sequence-based amplification, NGS—next generation sequencing metagenomics analysis, PCR-EIA—polymerase chain reaction-enzyme immunoassay, PCR-RFLP—nested polymerase chain reaction—restriction fragment length polymorphism, PCR-SSCP—a polymerase chain reaction and single-strand conformation polymorphism determination, PFIA—polarization fluorimetry, PRNT—a plaque reduction neutralization test, RFLP—restriction fragment length polymorphism or nucleotide sequencing, RT-PCR—a reverse transcription followed by a hemi-nested polymerase chain reaction, RT-LAMP—real-time reverse transcription-loop-mediated isothermal amplification assay, RT-PCR—reverse transcription—polymerase chain reaction, SNP—single nucleotide polymorphism, SPGA-EDTA buffer—a Sucrose-Phosphate-Glutamate-Albumin buffer containing ethylenediaminetetraacetate, STR-PCR—short tandem repeat polymorphisms chain reaction, QAGP—quantitative agar gel precipitation, qRT-PCR—quantitative reverse transcription—polymerase chain reaction, UHPLC-MS/MS—ultra-high-performance liquid chromatography—tandem mass spectrometry, WGA—whole genome amplification

usual way of biofluids or homogenate/suspensions sampling onto filter material is a dropwise application. A part of absorbing material, for instance, in a form of strip, was also used for biofluids saturation (Table 1). Tissues and similar specimens are usually applied on a carrier in a form of smears or crushes. Insect saliva or dry fecal spots are collected by a direct exposure of insects onto a filter paper. Nevertheless, whole blood as a source of specific antibodies or genetic material remains the most commonly used biofluid among all dried samples taken from animals so far (Table 1). At the same time, it should be noted that due to a big diversity of animal species and the tasks of particular investigation, the variety of described dried biosamples is much wider than in human medicine, where body fluids are usually utilized. Type of the used specimen is determined by the peculiarities of causative agent localisation in a body, for example, avian influenza virus can be found in parts of bird's body but not in blood, rabies virus – in brain tissue, infectious bursal disease virus – in bursa, etc. (Tables 1 and 2).

### Carriers for dried samples and sample pretreatment

Pure cellulose as an affordable and established material for DBS sampling has been rooted from neonatal screening starting from the 1960-s, and since then it was considered to be the standard sample carrier in most medical and scientific works (Demirev 2013; Meesters and Hooff 2013; Sharma et al. 2014). This type of membrane material was used in the majority of the reported veterinary and biological investigations too (Table 1). The investigations were carried out with cellulose absorbing filter paper (manufactured from 100% pure cotton) of various trademarks: mainly it was widespread Whatman (no.1, no.3, no.4, 3MM filter paper and others), less frequent it was Munktell, Schleicher&Schuell and some others, as well as blotting paper (Table 1). Some researchers used an ordinary filter paper or cone type paper typically used for coffee filters (Quintana et al. 2009) and even plain office and stationary paper (Maw et al. 2006; Suriyaphol et al. 2014) for drying and further it was used for PCR amplification of genetic material. Generally speaking, any porous cellulose or other absorbing material is sufficient to saturate whole blood or other biological fluids/specimens, and it allows to analyse the absorbed biomaterial at least qualitatively. The sampling method onto common papers can be suitable for surveys where laboratory resources are limited (Maw et al. 2006), but this type of material's applicability for the particular investigation should be evaluated (Suriyaphol et al. 2014). Commercial chemically treated cellulose-based sampling cards, such as protein saver card 903, FTA card, FTA elute card (Whatman) were used for sampling quite often (Table 1). Special FTA (Flinders

Technology Association) cards were developed to proceed cell lysis and protein denaturation while the biosample was applied onto the material and to preserve nucleic acids for subsequent DNA or RNA analysis. In case of PCR, FTA cards impregnated with detergents and other special substances are the most frequently used cards (Table 1). Moreover, they can provide virus inactivation, and are the safest to use among other Whatman filter papers (Wannaratana et al. 2021). Wannaratana et al. (2021) found that three Whatman cellulose carriers, namely FTA card, 903 card and qualitative filter paper grade 2 can preserve Marek's disease virus DNA for at least 30 days post spot but only on FTA cards no viable Newcastle disease virus was detected at all post spotted timepoints. For specific purposes, a common laboratory filter paper or stationary paper can be considered as affordable cost-efficient absorbing material in comparison with more expensive commercially available special sampling cards. For example, seeking for safe, inexpensive, simple and easy method of sample preparations, Maw et al. (2006) showed that ordinary paper with phenol fixation inactivates and sustains infectious bursal disease virus RNA as the FTA card. So, regardless of the paper quality, storage period and fixation method, viral RNA was consistently detected in all bursa dried imprints after 30 days storage at 37 °C. Under determination of avian sexing in DBS Suriyaphol et al. (2014) concluded that the most practical and cost-effective method across different types of commercial sampling cards, ordinary filter and stationery paper was the utilisation of Whatman grade 1 filter paper (ordinary filter paper) with the combination of methanol fixation and boiling.

It is well known that cellulose material used for whole blood absorption has some weak points, such as haematocrit effect and chromatographic effect, which are generally affecting the low molecular weight substances analysis and quantitative analysis (Velghe et al. 2019). These effects resulted from rheological properties of whole blood, hematocrit varying from sample to sample, and from the nature of cellulose material with hollow fibres, all together these factors influence the assay accuracy (De Kesel et al. 2013). That is why, along with other numerous approaches and devices for volumetric sampling of whole blood, the researchers consider the alternative non-cellulose materials for DBS preparations to minimize these effects. Some veterinary studies were performed with the use of nitrocellulose, nylon (Vilček et al. 2001; Wang et al. 2002) and fiberglass membranes (Samsonova et al. 2014, 2017, 2019a, b; Saushkin et al. 2016a, b, 2019). Just a few works were devoted to compare cellulose and non-cellulose membrane carriers for particular investigations. Vilček et al. (2001) compared cellulose, nitrocellulose and nylon carriers under the storage of dried whole blood and serum containing bovine viral diarrhea virus (RNA virus); the lowest yield of PCR products was obtained for classical



filtration paper. DBS stored on different carriers at +4 °C or -20 °C (up to 4 weeks) and at room temperature (up to 6 months) were stable. Wang et al. (2002) also compared cellulose, nitrocellulose and nylon carriers upon detection of duck hepatitis B virus in dried serum, and found that cellulose paper provides the highest yield of PCR products and nylon membrane provides the lowest.

All Whatman and similar cards are manufactured as a small piece of thick cellulose filter paper with special circled zones for a sample application. As reported, animal DMS were also collected with the help of some alternative sampling devices such as Tego Card (Yoon et al. 2010; Sun et al. 2013), Hemaspot device (Rosypal et al. 2014), isoCode stix (Jefferies et al. 2007) and Nobuto cellulose strips (Table 1). Nobuto cellulose stripes were first used in 1966 by Japanese scientists Kenzo Nobuto to collect swine blood for serodiagnosis of toxoplasmosis (Nobuto 1966). The strips consisted of two parts: the absorbent area and the distribution area. Later, Nobuto cellulose strips were found useful for wildlife sampling as a convenient tool for whole blood collection in a field, directly from a puncture or a wound (Table 1). Another type of stripes made of fiberglass and divided into equal zones for collecting and analysing of dried whole blood, serum or milk by ELISA and PCR were described (Samsonova et al. 2014, 2017, 2019a, b; Saushkin et al. 2016a, b, 2019). The proposed strip provides volumetric biofluid microsampling due to the properties of absorbing non-cellulose material consisted from solid fibers and the shape of the strip. This fiber glass strip has advantages over cellulose material in terms of mechanical strength, easiness of aliquoting of dried sample, small influence of sample hematocrit and uniform biofluid/analyte distribution across the strip (Samsonova et al. 2016, 2017, 2022; Saushkin et al. 2019). Based on similar principle a comb-shape 12-spot DBS card was designed that can be directly placed into ELISA plate wells for elution of antibodies (Knuutila et al. 2014). Moreover, a domestic device that can introduce eight blood combs simultaneously to the ELISA plate with no need for a separate elution/dilution step and speeding up the sample pretreatment was used. Overall, it should be highlighted that a strip of absorbing material can be considered as the most convenient way of whole blood sampling in the field so far, especially in hush conditions, with no need of dosing equipment. It is significant that in some works, the researchers, for the convenience of applying the blood sample, cut the cellulose material into strips for the possibility of impregnation of the membrane directly from puncture and ease of dosing in the subsequent stages (Aldo Gaggero and Suttmöller 1965; Lana et al. 1983; Avakian and Dick 1985; Roy et al. 1994, 1992; Stallknecht and Davidson 1992; Nogami et al. 1992; Reisen et al. 1994; Thangavelu et al. 2000; Dubay et al. 2006; Jefferies et al. 2007; de Oliveira et al. 2011; Randriamparany et al. 2014).

Analysis of the published works revealed that to perform serological tests the conditions of dried biosample extraction from cellulose carrier were chosen by authors empirically and usually included incubation of dried sample in a buffer solution from 1–2 h to overnight or even 24 h at room temperature or +4 °C. At the same time it was shown that 20–30 incubation under shaking at room temperature is enough to release antibodies from whole blood, serum or plasma dried on a glassfibre strip (Saushkin et al. 2016b, a, 2019; Samsonova et al. 2019a, b). Some works described the optimization of antibodies elution time and temperature (Hopkins et al. 1998; Chadio et al. 2002). Similarly, to increase concentration of cattle pregnancy-associated glycoproteins (PAGs) in paper eluate Sun et al. (2013) used a modified ELISA protocol including increased sample volume, longer incubation time and plate shaking during incubation. In general, there are no established standardized procedures for antibodies extraction from dried biological fluids. Antibodies have been extracted from a membrane carrier into different buffers such as borate buffer (Wolff and Hudson 1974; Sacks et al. 2002; Chomel et al. 2004; Bevins et al. 2016; Chandler et al. 2018), Tris buffer (Nogami et al. 1992; Chandler et al. 2018), physiological saline (Wolff and Hudson 1974; Kalayou et al. 2011), phosphate buffered saline (PBS) with detergent (Afshar et al. 1987, 1992; Roy et al. 1992; Hopkins et al. 1998; Holland et al. 2002; de Oliveira et al. 2011; Dam-Tuxen et al. 2014; Andersson et al. 2015; Al-Kappany et al. 2018) or PBS with no detergent (Aldo Gaggero and Suttmöller 1965; Platt and Adams 1976; Armstrong et al. 1991; Stallknecht and Davidson 1992; Cabrera et al. 1999; Mueller-Anneling et al. 2000; da Silva et al. 2000; Holland et al. 2002; Jordan et al. 2005; Dalerum et al. 2005; Mucker et al. 2006; Yoon et al. 2010; Dusek et al. 2011; Mercier et al. 2013; Rosypal et al. 2014; Abbott et al. 2014; Aston et al. 2014; Kamps et al. 2015; O'Hara et al. 2018; Santos et al. 2018; Ytrehus et al. 2021; Jennings-Gaines et al. 2021). Usually, the amount of blood absorbed and then retained from a filter paper was not assessed; however, that is an important issue during assay development or commercial ELISA kit adaptation for dried samples. Yu et al. (2007) established a linear equation between dry weight of filter paper and known volume of absorbed whole blood to estimate Sika deer blood volume absorbed by filter discs taken in the field. Aston et al. (2014) performed retrospective evaluation of several kinds of laboratory-grade filter paper along with commercial cellulose Whatman cards during anti-*T. gondii* antibody elution and detection from DBS taken from hunted ungulates. The dried blood concentration across different filter papers was determined by means of pipetting a predetermined volume of blood and calculating the area of the blood spot. To calculate dilution factor the authors modified agglutination assay protocol taking into account the number of punched disks (from blood spot) and

**Table 2** Storage stability studies for veterinary DMS

Analyte	*Dried sample spots or imprints	Carrier	**Maximum time of storage and storage conditions	Reference
<b>Antibodies</b>				
Antibodies (avian influenza virus)	Whole blood	Nobuto filter paper strip	3 months at RT	Dusek et al. 2011
Antibodies (Aujeszky's disease)	Whole blood	Whatman no.1 filter paper	1 year at 4 °C	Banks 1985
Antibodies (bovine herpesvirus-1)	Whole blood, serum	Whatman no.3 filter paper	8 months at 4 °C	de Oliveira et al. 2011
Antibodies (bovine leucosis)	Whole blood	Fiberglass strip	7 days at 37 °C; 24 h at 60 °C	Saushkin et al. 2019
Antibodies (foot-and-mouth disease)	Whole blood, serum	Blotting paper	60 days at RT	Aldo Gaggero and Suttmöller 1965
	Serum	Fiberglass strip	7 days 37 °C; 24 h at 60 °C	Samsonova et al. 2019b
Antibodies (Newcastle disease)	Whole blood	Whatman no.1 filter paper	3 weeks at RT	Roy et al. 1992
Antibodies (plague ( <i>Y. pestis</i> ))	Whole blood	Nobuto filter paper strip	454 days at -20 °C and 4 °C 40 days at RT	Bevins et al. 2016
Antibodies (toxoplasmosis)	Whole blood	Quantitative filter paper strips (Toyo Roshi Kaisha)	Up to 12 months (-80 °C to RT) with silica gel	Nogami et al. 1992
Antibodies (vesicular stomatitis)	Serum	Whatman 903 card	Up to 6 months (4 °C to RT)	Bolais et al. 2017
		Blotting paper	7 days at 37 °C; 1 day at 56 °C	Adams and Hanson 1956
Antibodies (visceral leishmaniasis)	Plasma	HemaSpot device	1 month at RT; 79% drop in 6 months at RT	Rosypal et al. 2014
Antibodies (brucellosis, neosporosis, West Nile virus disease, herpes, bovine parainfluenza, bovine respiratory disease; bovine viral diarrhea)	Whole blood	Nobuto filter paper strip	Up to 2 years at RT	Curry et al. 2014b
Antibodies (infectious bursal disease, infectious bronchitis, micoplasmosis)	Whole blood, serum	Fiberglass strip	7 days at 4 °C (serum); 7 days at 4 °C, RT, 37 °C; 60 days at 60 °C (blood)	Samsonova et al. 2019a
<b>RNA/DNA (infectious agents)</b>				
<b>Viruses</b>				
African swine fever virus (DNA)	Whole blood	FTA card	10 months under field conditions	Utenthal et al. 2013
		Whatman 3MM filter paper	9 months at RT 2 months at 37 °C	Randriamparany et al. 2014
Avian influenza virus (RNA)	Swab samples	FTA card	5 months at RT	Abdelwhab et al. 2011
	Cloacal and oropharyngeal swabs		Up to 30 days at RT for most subtypes	Keeler et al. 2012
	Cloacal and oropharyngeal swabs, lungs, spleen, kidneys and brain		150 days at -20 °C or RT	Jóźwiak et al. 2016
Bovine herpesvirus-1 (DNA)	Bovine semen	FTA card	28 days at 4–37 °C	Sarangji et al. 2018

Table 2 (continued)

Analyte	*Dried sample spots or imprints	Carrier	**Maximum time of storage and storage conditions	Reference
<b>Bovine leukaemia virus (DNA)</b>	Whole blood	Fiberglass strip	7 days at 37 °C; 24 h at 60 °C	Saushkin et al. 2019
Bovine respiratory disease complex (bovine viral diarrhoea virus (RNA), bovine respiratory syncytial virus (RNA), bovine coronavirus (RNA), bovine herpesvirus-1 (DNA))	Diagnostic swab fluids, deep nasal swabs	Filter paper (Toyo Roshi Kaisha) FTA card	At least 10 days at RT Up to 14 days at -27 °C to 46 °C	El Daous et al. 2020 Liang et al. 2014
<b>Bovine viral diarrhoea virus (RNA)</b>	Whole blood, serum	Whatman no.1 filter paper, nitrocellulose no.71002, HYBOND-M nylon	Up to 4 weeks at -20 °C, 4 °C and 6 months at RT	Vilček et al. 2001
<b>Duck hepatitis B virus (DNA)</b>	Serum	Whatman no.1 filter paper	4 weeks at -70 °C and -20 °C; decreased yield at temp. more than 4 °C	Wang et al. 2002
<b>Foot-and-mouth disease virus (RNA)</b>	Cell culture isolates, tongue epithelium	FTA card	80–120 days	Muthukrishnan et al. 2008
<b>Fowl adenovirus I (DNA)</b>	Cell culture isolates, tongue and foot epithelium	FTA card	22–56 days at 21–45 °C and relative humidity 20–100%	Madhanmohan et al. 2016
<b>Infectious bronchitis virus (RNA)</b>	Cell culture isolates, tongue epithelial suspension and impression smears Liver Tracheal swabs	FTA card FTA card	Up to 6 weeks at 4–37 °C	Biswal et al. 2016
<b>Infectious bursal disease virus (RNA)</b>	Tissues and swab samples	FTA card	198 days at -20 °C	Moscoso et al. 2007
<b>Hemorrhagic enteritis virus (DNA)</b>	Water solution, spleen smears (HEV)	Hybond C nitrocellulose filter paper	Up to 15 days at 4 °C and RT, slight decrease at 41 °C	Moscoso et al. 2005
<b>Infectious bursal disease virus (RNA)</b>	Bursa	FTA card	Up to 21 days at up to 40 °C (partial sequencing); Up to 14 days at 4 °C and RT (full sequencing)	Manswr et al. 2018
<b>Marek's disease virus (DNA)</b>	Blood, solid tumors Vaccine	Chromatography paper, filter paper, stationery copy paper, FTA card FTA card FTA card, 903 card, qualitative filter paper grade 2 (all Whatman)	5–30 days at RT or 37 °C	Pitcovski et al. 1999
			15 days at RT, 8 months at -20 °C; 1–3 months at 25 °C and at least 8 months at -20 °C for RFLP or sequencing	Moscoso et al. 2006
			30 days at 37 °C	Maw et al. 2006
			8 months at RT	Cortes et al. 2009
			Up to 30 days at RT	Wannaratana et al. 2021



Table 2 (continued)

Analyte	*Dried sample spots or imprints	Carrier	**Maximum time of storage and storage conditions	Reference
<b>Metapneumovirus (RNA)</b>	Turbinate, trachea and lung	FTA card	Up to 60 days at 4 to 6 °C	Awad et al. 2014
<b>Newcastle disease virus (RNA)</b>	Trachea, lung, caecal tonsil and cloacal faeces	FTA card	15 days at RT	Perozo et al. 2006
<b>Peste des petits ruminants virus (RNA)</b>	Caecal tonsils, kidney, proventriculus, spleen, trachea, faecal swabs and intestinal lesions		Up to 30 days at 4 °C and RT	Narayanan et al. 2010
<b>African swine fever virus (DNA)</b>	Whole blood	Whatman 3MM filter paper, FTA card	3 months at up to 32 °C (RNA); 9 months at up to 37 °C (DNA)	Michaud et al. 2007
<b>Peste des petits ruminants virus (RNA)</b>	Whole blood, nasal swabs	Whatman 3MM filter paper	16 months at -70 °C 7 days at RT (molecular detection and genotyping)	Bhuiyan et al. 2014
<b>Porcine reproductive and respiratory syndrome virus (RNA)</b>	Whole blood, serum, organs (lung, tonsil, and superficial inguinal lymph nodes), oral fluid	FTA card	14 days at 4 °C and RT	Linhares et al. 2012
<b>Swine influenza virus (RNA)</b>	Allantoic fluid, nasal swabs, lung tissue	FTA card	7 days at RT	Maldonado et al. 2009
<b>Rabies virus (RNA)</b>	Brain tissue	no. 903 filter paper; Schleicher and Schuell	222 days at RT	Wacharaphesadee et al. 2003
<b>Ross River virus (RNA)</b>	Virus isolates	FTA card	35 days at RT	Picard-Meyer et al. 2007
<b>Fish betanodavirus (RNA)</b>	Brain tissue		3 months at -80 °C and -20 °C; degradation of vRNA at 4 °C and RT storage	Sakai et al. 2015
<b>Other infectious agents (DNA)</b>	Brain tissue	Whatman 903 filter paper	Up to 2 years at RT	Rasolonjatovo et al. 2020
<b>Babesia gibsoni (DNA)</b>	Mosquito saliva	Honey-soaked FTA card, filter paper	28 days at RT	Hall-Mendelin et al. 2010
<b>Micoplasma (M. gallisepticum and M. synoviae) (DNA)</b>	Tissue homogenate, cell culture supernatant, gonadal fluid-milt and seawater spiked with betanodavirus	FTA card	28 days at 4 °C	Navaneeth Krishnan et al. 2016
<b>Trypanosoma species (DNA)</b>	Whole blood	Whatman 3MM filter paper	2 months at RT	Tani et al. 2008
	Tracheal swabs	FTA card	60 days at 4–40 °C	Moscoso et al. 2004
	Buffy coat	Whatman no.3 filter paper	5 months at RT; 2 years at -20 °C (results not shown)	Geysen et al. 2003
<b>Streptococcus agalactiae (DNA)</b>	Milk	Xinhua grade 3 chr paper treated with EDTA	Up to 4 weeks at RT and 37 °C	Wu et al. 2008

Table 2 (continued)

Analyte	*Dried sample spots or imprints	Carrier	**Maximum time of storage and storage conditions	Reference
<b>Other DNA investigations</b>				
Aphid specimens collection and storage (DNA)	The purified DNA or the solution of the cell lysis buffer	Filter Paper Qualitative P5 (Thermo Fisher Scientific), FTA card	Up to 2 years	Miller et al. 2013
Avian DNA	Whole blood	FTA card	Up to 4 years	Smith and Burgoyne 2004
Chicken mites ( <i>Dermanyssus gallinae</i> ) (DNA)	Mite homogenate	FTA card	1 year at RT	Desloire et al. 2006
Genotyping of casein A1/A2 variants (DNA)	Milk	Whatman no.3 filter paper treated with SPGA-EDTA buffer	Up to 1 month at -80 °C, -20 °C, RT	Venkatesh and Gopal 2018
Porcine DNA	Whole blood	FTA card	Up to 3 years	Fowler et al. 2012
<b>Other substances</b>				
Cyclosporin A	Whole blood	Whatman 903 card	9 days at RT in the dark under a dry atmosphere	Mohamed et al. 2012
Enrofloxacin, ciprofloxacin	Whole blood	Filter paper	4 weeks at -20 °C, 4 °C and RT	Posyniak et al. 2002
Fatty acids	Whole blood	PerkinElmer spot saver card	1 year at -80 °C	Wood et al. 2021c
Pregnancy-associated glycoproteins (PAGs)	Whole blood	TEGO card	14 days at 4 °C; 22–34% reduction upon storage at RT (14 days)	Sun et al. 2013
Progesterone	Whole blood	Schleicher and Schuell 2992 filter paper	Several weeks at -20 °C, RT and 4 °C; Up to 30% drop after 3 months	Chadio et al. 2002
Lysosomal enzymes	Milk	Fiberglass strip	1 month at 4 °C; 7 days at 37 °C; 24 h at 60 °C	Samsonova et al. 2014, 2017
Mercury	Whole blood	Munktel TFN 06–079 card	6 months at 4 °C; 20% reduction of enzyme activity in 1 year storage at RT (except $\beta$ -glucuronidase)	Sewell et al. 2012
Toxic heavy metals (As, Se, Hg, Pb)	Whole blood	Whatman 903 card	Up to 1 year at -20 °C to RT; 20 min at 100 °C;	Perkins and Basu 2018
50 compounds including anabolic steroids, $\beta$ 2-adrenoceptor agonists, corticosteroids, HIF-1 stabilizers, PPAR $\delta$ agonists, SARMS, and aromatase inhibitors	Whole blood	Whatman 903 card	Up to 180 min at 56 °C; 3 months at RT to 30 °C up to 80% humidity	Lehner et al. 2013
		Whatman 903 card	24 months at -20 °C	Moeller and Yang 2021
		Whatman 903 card	87 days at RT and 30–60% humidity	
			The majority of substances showed good stability (> 50% of initial concentration)	

\*HEV—hemorrhagic enteritis virus, HIF-1—hypoxia inducible factor-1, PPAR—peroxisome proliferator-activated receptor, SARM—selective androgen receptor modulators

\*\*RT – room temperature

the elution buffer volume. This method allows researchers to adapt their approaches to different available absorbing materials. Fiber glass material in a form of a strip allows the calculating of predetermined sorption capacity per a single square piece of a strip, so the development and assessment of the target analyte extraction efficiency is an easier task than for cellulose material (Samsonova et al. 2016, 2017, 2019b; Saushkin et al. 2019).

Nucleic acids extraction from DMS was performed with the use of most common DNA/RNA extraction methods. For example, Cardona-Ospina et al. (2019) summarized RNA extraction from FTA cards used for detection and characterization of viral RNA pathogens from fieldwork. Commercially available DNA extraction kits can be optimized to provide the most accurate and precise recovery for molecular epidemiological studies as it was shown for *Trypanosoma cruzi* DNA extracted from canine and skunk DBS (Gulas-Wroblewski et al. 2021). The issue of the best practice for preparation of samples from FTA cards for the molecular diagnosis under large-scale epidemiological studies was addressed in the work of Ahmed et al. (2011) using African trypanosomes as a model object. The authors found that to improve the detection of trypanosomes by PCR, DBS should better be eluted from cards using Chelex®100 than directly applied into PCR. The authors also emphasized that to overcome any problems associated with uneven distribution of parasite DNA on the card matrix and to increase the sensitivity of the molecular diagnosis of trypanosome infections the increased numbers of discs cut from DBS should be used. Seeking for the most effective high-quality unfragmented DNA extraction method for genomic studies Love Stowell et al. (2018) compared three types of paper (Nobuto filter paper strip, FTA card, FTA Elute card) and four commonly used extraction methods with some modifications using ungulate and canine dried whole blood. The authors concluded that preservation FTA cards were not adequate for providing unfragmented DNA for downstream genomic applications such as microsatellite and genomic sequencing especially for mammals which have non-nucleated blood cells. However, other researches successfully used FTA cards for genetic applications such as microsatellite genotyping (King et al. 2005; Lucentini et al. 2006; Carr and Appleyard 2008; Heim et al. 2012; Nunziata et al. 2016). For example, Lucentini et al. (2006) described a nondestructive method for obtaining the high-quality DNA from fish body mucus and buccal cells, making it the best choice for populational genotyping. In another investigation FTA cards was shown to be a good source of high-quality abalone DNA particularly for mitochondrial DNA and nuclear microsatellite amplifications for abalone (Carr and Appleyard 2008).

The separation of a part of the dried biospecimen spot on a membrane is usually carried out using a puncher, obtaining one or multiple paper disks of small diameter. Using of a

puncher for DNA/RNA samples can be associated with cross contamination, which demands additional cleaning step and blank cards punching (Linhares et al. 2012). At the same time, the use of non-cleaned and cleaned scissors showed no false positive results, and provided correct amplicon sizes (Suriyaphol et al. 2014). It was also reported, that the standard protocol of punching a membrane disk with dried blood sample leads to strong cross-contamination, despite cleaning the puncher with alcohol and using sterile wipes (Jefferies et al. 2007). The authors sequentially punched sterile filter paper, purified by alcohol after a positive sample; and obtained six potentially contaminated disks. Further, these disks were subjected to the standard procedure of DNA extraction and amplification; it showed that a false-positive result could be obtained even though the third-punched disk of sterile paper was used.

Extraction methods for other substances depended on the extractant type, thus enzymes and other proteins were usually extracted into buffers whereas for the low molecular substances organic solvents were preferably used. PAGs were extracted into PBS for 1 h at room temperature (Sun et al. 2013) and cholinesterase was extracted with the water solution of Triton X-100 (Trudeau et al. 2007). Progesterone was extracted from dried blood water eluate with the use of diethyl ether (Lin et al. 1988), from DBS—into PBS supplied with bovine serum albumin (Chadio et al. 2002), or alternatively it was released from dried milk during immunological reaction (Samsonova et al. 2014, 2017). To recover antibiotic enrofloxacin and its metabolite ciprofloxacin from dried blood Posyniak et al. (2002) found that among the investigated eluents the best one was acetonitrile. Moreover, the eluate ultrasonication showed preferences over shaking in terms of better extraction and protein denaturation. Acylcarnitines from dried serum spots were extracted with methanol (Sander et al. 2018; Worku et al. 2021).

To summarize it all, it should be emphasized that the choice and evaluation of a filter paper or a sampling card from a variety of available items is determined by a specific goal and optimal criteria for a particular task. An extraction procedure should be optimised to keep extractant level as high as possible, preferably close to that of the corresponding liquid sample, as it was shown for DNA/RNA extraction protocols from engorged and unfed individual mites (Desloire et al. 2006), from canine DBS (Tani et al. 2008) and from dried brain tissue (Sakai et al. 2015).

## Methods and detected substances

Veterinary, biological and ecological investigations, based on DBS technology, are supported by a range of immunochemical, genetic and instrumental analytical methods. Veterinary diagnostics with the use of DMS are based on

the results of a variety of methods, including serological methods for the specific antibodies detection and PCR to reveal pathogen genetic material (Table 1). Serological methods for filter paper eluates include virus neutralization test, methods based on precipitation of antigen–antibody complex in agarose gel, indirect fluorescent antibody test, a variety of agglutination tests, radioimmunoassays, immunochromatographic test, and multiple variants of ELISA (Table 1). ELISA was also used to detect progesterone in cows' dried milk and pigs' dried blood (Lin et al. 1988; Chadio et al. 2002; Samsonova et al. 2014, 2017) and PAGs in cows' dried blood (Sun et al. 2013) as pregnancy markers. A variety of PCR methods and its modifications used for DMS eluate amplification are vast, and included nested PCR, a hemi-nested PCR, real-time reverse transcription-loop-mediated isothermal amplification assay (RT-LAMP), the low-stringency single specific primer PCR (LSSP-PCR), and others (Table 1). Amplified fragment length polymorphism—AFLP, nested polymerase chain reaction – restriction fragment length polymorphism—PCR–RFLP, nucleic acid sequence-based amplification—NASBA, sequencing, DNA barcoding and other techniques were employed for identification and confirmation of disease causative agent, or investigations of gene polymorphism, genotyping, phylogenetic analysis and population genetic studies (Table 1).

Instrumental analytical methods, such as high performance liquid chromatography (HPLC), inductively coupled plasma mass spectrometry (ICP-MS), liquid chromatography (LC), mass-spectrometry (MS), atomic absorption spectrometry (AAS), and others were used for detection of pesticides and toxic environmental contaminants in wild birds (Shlosberg et al. 2011, 2012; Lehner et al. 2018), antibiotics in chickens (Posyniak et al. 2002), toxic heavy metals in wild birds and animals (Shlosberg et al. 2011, 2012; Lehner et al. 2013; Hansen et al. 2014; Perkins and Basu 2018; McHuron et al. 2019), cyclosporine A in cats (Mohamed et al. 2012) and doping agents in horses (Moeller and Yang 2021). Lyosomal enzyme activity in cats and dogs was estimated by fluorescent method (Sewell et al. 2012), cholinesterase activity as function of its inhibitors (as organophosphate and carbamate insecticides) in wild birds was performed by spectrophotometric method (Trudeau et al. 2007), blood meal identification in mosquitos was done by MS spectra (Niare et al. 2017).

## Dried veterinary samples storage stability

Remote blood sampling for diagnostics and scientific investigations should be supported by the analytes stability in dried biological matrix, shipped from the farm/field to the laboratory and then stored until needed. In veterinary works, storage stability issue has been addressed in relation to the

recovery of antibodies, nucleic acids and other substances across a variety of the DMS specimens (Table 2). Paper-immobilised antibodies usually showed good storage stability and temperature and humidity resistance probably due to their relatively rigid three-dimensional structures (Wang et al. 2012) and their quite high concentration and protein surroundings in dried blood. In veterinary diagnostics high storage stability of antibodies was described for dried whole blood, serum and plasma (Adams and Hanson 1956; Karstad et al. 1957; Benson and Mickle 1964; Aldo Gaggero and Suttmöller 1965; Stallknecht and Davidson 1992; Dubay et al. 2006; Curry et al. 2014a; Nemeth et al. 2017, 2021). Majority of the antibodies stability studies were performed on common filter paper and fiber glass stripes (Table 2). Curry et al. (2014b) showed good long-term antibodies stability (up to 2 years at ambient conditions) in reindeer and caribou DBS collected on Nobuto filter paper stripes in the field. Interestingly, better storage stability of chicken immunoglobulins stored on fiber glass strip was observed for dried whole blood than for dried serum samples (Samsonova et al. 2019a).

Many investigators examined viral and other causative agents storage stability (RNA or DNA) on sampling cards, mostly on nucleic acids preservation cards like FTA, or other absorbing materials (Table 2). Thus, Pitcovski et al. (1999) used two non-enveloped viruses, infectious bursal disease virus (IBDV, RNA virus) that infects chickens or hemorrhagic enteritis virus (HEV, DNA virus) that usually affects turkeys as model objects that were applied onto nitrocellulose membrane in a water solution or within spleen smears (HEV). It was shown that viruses could be stored in dried form on paper for 5–30 days at room temperature or 37 °C, so this method permits the storage of viral samples and shipping them from the field to a laboratory. Viral RNA was found to remain stable in dried samples for different avian viruses such as Newcastle disease virus (Perozo et al. 2006; Narayanan et al. 2010), infectious bronchitis virus (Moscoso et al. 2005; Manswr et al. 2018), infectious bursal disease virus (Moscoso et al. 2006; Maw et al. 2006), avian influenza virus (Abdelwhab et al. 2011; Keeler et al. 2012; Józwiak et al. 2016) and avian metapneumovirus (aMPV) (Awad et al. 2014). It was possible to perform molecular characterization of both subtypes A and B aMPV, with the use of inoculated FTA cards stored for up to 60 days at 4 °C to 6 °C (Awad et al. 2014). Among livestock RNA viruses investigated included rabies virus (Wacharapluesadee et al. 2003; Picard-Meyer et al. 2007; Sakai et al. 2015; Rasolonjatovo et al. 2020), foot-and-mouth disease virus (FMDV) (Muthukrishnan et al. 2008; Biswal et al. 2016; Madhanmohan et al. 2016), Peste des petits ruminants virus (PPRV) (Michaud et al. 2007; Bhuiyan et al. 2014), porcine reproductive and respiratory syndrome virus (Linhares et al. 2012), Ross river virus (RRV) (Hall-Mendelin et al. 2010),

swine influenza virus (Maldonado et al. 2009), bovine viral diarrhoea virus (Vilček et al. 2001; Liang et al. 2014), bovine respiratory syncytial virus and bovine coronavirus (Liang et al. 2014). Thus, rabies virus was confirmed to be stable within dried brain tissue stored on filter paper up to 2 years at room temperature (Wacharapluesadee et al. 2003; Rasolonjatovo et al. 2020). However, Sakai et al. (2015) reported degradation of viral RNAs, which occurred after storage of dried brain tissue on FTA card at 4 °C and room temperature, whereas viral RNA was stable over 3 months on the cards stored at –80 °C or –20 °C. It seems that nucleic acid preservation cards impregnated with special lysing and denaturing substances (FTA cards) were not the best choice for long-term storage of rabies virus. Bhuiyan et al. (2014) revealed PPRV virus successful storage on filter paper at -70 °C for 16 months and at RT for 7 days to perform molecular detection and genotyping. Hall-Mendelin et al. (2010) reported that both honey-soaked FTA cards and untreated filter paper cards were able to bind RRV RNA from mosquito saliva and preserve it at 23 °C for at least 28 days. Natarajan et al. (2000) demonstrated the usefulness of FTA cards for the preservation of mammalian and plant specimens for subsequent RNA analysis. Summarising literature data of the reported effectiveness of FTA cards for storage and shipment of viral RNA, Cardona-Ospina et al. (2019) concluded that the cards could be a suitable option for these purposes in areas where proper conditions for RNA preservation are difficult to achieve. Different DNA viruses usually demonstrated good storage stability in dried samples, for example, avian Marek's disease virus (Cortes et al. 2009), duck hepatitis B virus (Wang et al. 2002) and fowl adenovirus I (Moscoso et al. 2007), bovine herpesvirus-1 (Sarangi et al. 2018) and bovine leucosis virus (Saushkin et al. 2019; El Daous et al. 2020), African swine fever virus (Michaud et al. 2007; Uttenthal et al. 2013; Randriamparany et al. 2014). Common filter papers are cheap and could be as good as more expensive commercial FTA cards for virus storage, like it was shown for African swine fever virus (Uttenthal et al. 2013; Randriamparany et al. 2014), infectious bursal disease virus (Moscoso et al. 2006; Maw et al. 2006) and Marek's disease virus (Cortes et al. 2009; Wanaratana et al. 2021). In another investigation, FTA cards and Whatmann 3MM filter paper (cheaper option) showed similar results for DBS long-term storage (up to 9 months) at ambient and tropical temperature (22, 32 and 37 °C) to detect RNA and DNA containing viruses (Michaud et al. 2007).

DNA of other causative agents such as *Mycoplasma* (Moscoso et al. 2004), *B. gibsoni*, a protozoan parasite (Tani et al. 2008), *Trypanosoma*, unicellular parasitic flagellate protozoa (Geysen et al. 2003) and *S. agalactiae*, a common veterinary pathogen (Wu et al. 2008) were also investigated in terms of DNA storage stability on filter paper. FTA cards

was shown to be an excellent tool for long-term DNA storage and archiving (up to 44 months) for avian (Smith and Burgoyne 2004) and porcine DBS (Fowler et al. 2012). Fowler et al. (2012) showed that DNA extracted from FTA cards was of good quality to amplify the whole genome and to perform the meaningful single nucleotide polymorphism chip studies even after three years of blood spotting. Venkatesh and Gopal (2018) showed that dried milk could be stored for up to a month at room temperature for the further detection of A1/A2 variants of *b*-casein using PCR.

Some other high and low molecular weight substances recovery was checked during storage studies of dried samples (Table 2). PAGs were stable for 14 days at 4 °C in dried blood, whereas storage at room temperature caused 22–34% reduction (Sun et al. 2013). Lysosomal enzymes activity did not change much after DBS storage at 4 °C for 6 months; however, 1 year storage at ambient room temperature (20 °C) caused about 20% reduction of activity for all investigated enzymes, except  $\beta$ -glucuronidase (Sewell et al. 2012). Antibiotic enrofloxacin and its metabolite ciprofloxacin were stable in dried blood samples for 4 weeks at -20 °C, +4 °C or room temperature (Posyniak et al. 2002). Mohamed et al. (2012) demonstrated immunosuppressant cyclosporine A stability in dried blood spots, stored for 9 days, placed at room temperature in the dark and dry environment. A steroid hormone progesterone in dried whole blood showed a good stability after storing for a few weeks at +4 °C and -20 °C. However, 30% drop of recovered concentration was observed in 3 months (Chadio et al. 2002). At the same time progesterone recovery from dried milk was stable while samples were kept at +4 °C (1 month), room temperature (1 month), +37 °C (1 week) or +60 °C (24 h) (Samsonova et al. 2017). For fatty acid analysis, animal DBS samples should be stored at -80 °C to maintain stability, which limit their usefulness for field sample collection and shipment (Wood et al. 2022). As it was shown previously in numerous publications on human DBS, to maintain fatty acids storage stability at higher temperatures, and to provide dried samples delivery to a laboratory at ambient temperatures, the sampling cards should be impregnated with stabilising agents in order to prevent oxidative destruction of polyunsaturated fatty acids (Liu et al. 2014). It was shown that chemical elements (Hg, As, Se and Pb) recovery from dried blood samples did not differ much over 1–2 year storage (Lehner et al. 2013; Perkins and Basu 2018). Moeller and Yang (2021) showed that the majority of 50 investigated substances (anabolic steroids,  $\beta$ 2-adrenoceptor agonists, corticosteroids, hypoxia inducible factor-1 stabilizers, peroxisome proliferator-activated receptor  $\delta$  agonists, selective androgen receptor modulators, and aromatase inhibitors), which can be used as doping agents for horses, were quite stable after almost 3 months of DBS storage at ambient temperature.



Dried filter paper samples shipping, as well as wildlife sample collection in the field, can face sampling conditions, temperature, humidity and other changes, and that can potentially effect the sample preservation and quality. Madhanmohan et al. (2016) investigated FTA cards impregnated with FMDV RNA (tongue and foot epithelium samples), collected and posted for long route between cities under ambient conditions during post-monsoon and summer seasons in India. Irrespective of adverse atmospheric temperature (21–45 °C) and relative humidity (20–100%), FMDV genome or serotype could be identified in all samples (22–56 days post-collection). Tissues sampling onto cards can arise issues of oversampling; it can also lead to the necessity of robust protocols for humidity control and tissue sample application in the field (Borisenko et al. 2008). In this matter, the authors did not recommend liver as the tissue source for FTA sampling to recover DNA barcodes. Love Stowell et al. (2018) stressed that biological samples preservation from rare and elusive wildlife species for genomic studies poses many challenges. To prevent contamination and DNA degradation in unfavourable sampling conditions, there should be an ability to dry filter paper samples quickly and completely. The authors discovered quick molding of Nobuto filter paper stripes, even when stored with desiccants, and had concerns that the high yield of total DNA from these stripes was the result of the contamination. On the base of massive collection of DBS samples from wildlife for use in serologic testing, Curry et al. (2014b) evaluated dried samples performance under simulating of potential challenges. The challenges comprised different storage durations and different processing/storage regimes, including freezing or drying. Thus Curry et al. (2014b) summarized key recommendations for collection, processing, storage, and shipping of Nobuto filter strips. The authors recommend dry storage at room temperature or at 4 °C up to 2 years, and for longer periods – storage in freezed condition was recommend. Perkins and Basu (2018) investigated laboratory prepared DBS across a range of temperature, humidity, storage duration changes and showed that mercury concentrations for whole blood and DBS were significantly correlated.

The published results on animal DMS shipping and storage revealed that in most cases dried samples with target analytes, including RNA viral agents, could be transported to a laboratory at ambient conditions or even elevated temperature (37 °C) without lack of stability for 7 days at least, and quite often can be stored for much longer. However, as it was pointed out by some authors, humidity is probably the most critical issue for dried samples storage. At the same time, in spite of humidity and temperature changes under samples shipping and storage, successful stability cases were described, and, at the end of the day, the resistance to surrounding conditions depends of the target analyte's nature. Anyway, the dry storage is essential to prevent molding

and potential membrane/sample contamination. And in this regard, alternative, not natural materials, such as polymer or fiber glass carriers that can survive possible biodegradation, should be taken into consideration.

## **DBS samples in veterinary medicine and animal biology: diagnostics, monitoring, surveillance, genetic investigations**

The main applications of DBS technology in veterinary and biology are disease diagnostics, sero-surveillance and genetic investigations. DMS specimens, collected of different animal species, provide rapid sample throughput suitable for large-scale epidemiological investigations, which are supported by serological screening methods and PCR. The use of DBS technology is promising for infectious diseases diagnosis, genotyping and sero-surveillance in livestock and poultry and for the post-vaccination control for serum antibodies. Livestock and poultry air-dried biosamples on filter paper were used for monitoring and diagnosis of foot-and-mouth disease, enzootic leucosis, bluetongue disease, trypanosomosis, brucellosis in cattle, toxoplasmosis and Peste des petits ruminants virus disease in small cattle, Aujeszky disease, porcine reproductive and respiratory syndrome, African swine fever in pigs, avian influenza, Newcastle disease, infectious bronchitis, infectious bursal disease in chickens, and for many others (Table 1). Diagnostic work was in close connection with causative virus geno/serotyping and phylogenetic analysis either (Michaud et al. 2007; Madhanmohan et al. 2013; Józwiak et al. 2016; Ball et al. 2016; Gohariz et al. 2017; Manswr et al. 2018). In veterinary studies, much attention was paid to epidemiological surveys of the vector-transmitted animal trypanosomosis prevalence, based on DBS samples (Table 1). Trypanosomosis is an acute concern in African countries that requires reliable surveys of the disease distribution along with causative *Trypanosoma* species identification (Adams et al. 2006; Brito et al. 2008). DBS can be more effective than liquid samples for *Trypanosoma* identification among infected and uninfected animals, with use of the serological method (Hopkins et al. 1998). The authors noted that obtaining serum in the field for the native (liquid) sample standard analysis requires much more effort, since it is necessary to take a significant amount of blood from the caudal or jugular vein. Holland et al. (2002) demonstrated a lower cut-off value, a higher specificity and sensitivity while using eluted blood spots in an antibody ELISA detection for tsetse transmitted trypanosomosis. In general, the use of DBS approach in tropical regions, hard-to-access and remote areas is of great practical interest. Epidemiological surveys of the prevalence of tsetse-transmitted bovine trypanosomosis requires reliable surveys of the disease distribution (Smit et al. 2014). Many wild species can

serve as *Trypanosoma* reservoir, so susceptible livestock is at risk to be infected by transmission via vector insects (tsetse flies, triatomines and others), while being close to areas inhabited with wildlife species. Therefore, collection and analysis of DBS samples from wild and domestic animals allows assessing the disease prevalence and distribution area (Ventura et al. 2002; Herrera et al. 2005). In this connection, *Trypanosoma* vector insects are the source of parasite genetic material and the investigation specimen in the form of dried midgut (Boid et al. 1999; Adams et al. 2006; Gillingwater et al. 2010) or dry fecal spots (Russomando et al. 1996; Machado et al. 2000; Dorn et al. 2001; Brito et al. 2008; Braz et al. 2008).

Dried samples were also used to purposes not associated with livestock infectious diseases, such as pregnancy diagnosing in pigs (Lin et al. 1988; Chadio et al. 2002) and cows' progesterone profile (Samsonova et al. 2014, 2017), antibiotic detection in chicks (Posyniak et al. 2002) and avian sexing (Suriyaphol et al. 2014). Dried milk spots were used to detect mastitis pathogens (Wu et al. 2008; Durel et al. 2015). Venkatesh and Gopal (2018) used dairy cows' dried milk and whole blood spots for casein genotyping (A1/A2 variants of  $\beta$ -casein). Evaluation of the nutritional status of ranging dairy cows (zebu) was based on the amino acids and acyl carnitines detection in dried serum spots (Worku et al. 2021). Sander et al. (2018) detected acylcarnitines accumulation in dried serum spots as a result of inhibited  $\beta$ -oxidation of fatty acids, that was atypical myopathy indication in horses, caused by some maple trees seeds or seedlings ingestion.

According to the published works among all infectious diseases in pets, the main attention was paid to the detection of rabies and canine visceral leishmaniasis in dogs' dried brain and blood spots, toxoplasmosis in cats' DBS (Table 1). In Brasil canine visceral leishmaniasis represents a serious public health problem due to its wide distribution and severity of its clinical forms. Extensive epidemiological surveys of the disease supported with native and dried sample collection and analysis were performed (Braga et al. 1998; Cabrera et al. 1999; da Silva et al. 2000; Cortes et al. 2004; Palatnik-de-Sousa et al. 2004; Nunes et al. 2007; Figueiredo et al. 2010a, b). Domestic dogs usually live close to humans and livestock, so they can participate in zoonoses transmission. Epidemiologic studies help to assess the exposure to particular vector-borne pathogens in rural dogs and associated ticks, determine their prevalence, characterize the pathogens with the help of molecular methods and evaluate the risk that these pathogens pose to humans and wildlife (Proboste et al. 2015). DBS samples of dogs and cats were also used for enzymatic diagnosis of lysosomal storage disease (Sewell et al. 2012) and for cyclosporine A detection to control of immune-mediated and allergic disorders (Mohamed et al. 2012).

DBS technology is a very valuable approach to monitoring of wildlife animals diseases. Epidemiological studies usually include the detection of the pathogens presence and the pathogens prevalence determination in animals and/or associated vectors. The sero-surveillance results help to assess the actual infection prevalence in a particular animal population and to predict future epizootics in wildlife and exposure risk for humans, if appropriate. Many wildlife infectious diseases are poorly controlled. Due to the complexity of mass sampling in one day, the probes can be collected and accumulated for a long period. In this matter, hunter- or trapper-harvested animals have been a useful resource for sample collections in wildlife health studies (Yu et al. 2007; Sintasath et al. 2009a, b; Curry et al. 2011; Aston et al. 2014). During this time, the point of samples storage or transportation can arise especially in harsh environment conditions including high humidity, low or very high temperature. Liquid samples need to be frozen, which is a complicated task under field conditions, moreover the transportation of a single sample is unjustified. DBS technology allows solving the problem because the dried samples can be stored throughout the expedition under ambient conditions.

Published works comprise a vast range of diseases monitored in wildlife, including trypanosomosis, plague, brucellosis, toxoplasmosis and many others (Table 1). Brucellosis is difficult to control, for instance, in Arctic caribou and reindeer population in Canada due to the harsh climate and the huge habitat (Curry et al. 2011, 2014b). The DBS approach was used for reindeer brucellosis monitoring: DBS samples were taken onto Nobuto strips from deer killed during the shooting under extreme conditions at temperatures up to  $-40$  °C and then analysed by ELISA (Curry et al. 2011). Livestock are susceptible to this disease either, and the main cause of infection is transmission from wildlife. In addition, human infection danger is not excluded due to insufficient heat treatment of food. Wildlife animals can be resistant to clinical disease, for instance, coyotes are resistant to bacterium *Y. pestis*, causative agent of plague (Abbott et al. 2014), but develop anti-pathogen antibodies, by that means providing a way to monitor the particular disease activity in a region. Carnivores are employed in sero-surveys as sentinel animals due to multiple contact with various rodent species, which resulted in infection with *Y. pestis* via their fleas (Chandler et al. 2018). Similarly, wild boar was used as sentinel species during large-scale bovine tuberculosis surveys (Santos et al. 2018). Wild animals can also be a host of the severe human infections causative agents. Thus, occurrence of Puumala virus in wild bank voles which causes febrile illnesses in humans as well as hantavirus antibodies prevalence assessment and the virus genetic variability studying was done with the help of Nobuto strips whole blood samples (Ahlm et al. 1997; Alexeyev et al. 1998; Olsson et al.

2003; Johansson et al. 2008). West Nile virus, (WNV, one of arboviruses spread by an arthropod) has become endemic in North America since 1999 and it is maintained through enzootic transmission between birds and mosquitoes. Humans and other mammals also can be infected after the bite of an infected mosquito. Monitoring pattern with dead bird clusters (dried oral swab samples) (Foss et al. 2016) and serological surveys of WNV-specific antibody prevalence in wild birds (DBS), are the two main ways for tracking WNV activity (Sullivan et al. 2006; Nemeth et al. 2017, 2021). Mosquitos are the vector insects, which can expectorate viruses in their saliva during sugar feeding. Mosquito saliva dried sample collection on honey-soaked FTA cards is used for arboviruses and other viruses surveillance programs that provide information of viral prevalence and distribution (Hall-Mendelin et al. 2010; Ritchie et al. 2013; van den Hurk et al. 2014; Flies et al. 2015; Johnson et al. 2015; Burkett-Cadena et al. 2016; Kurucz et al. 2014, 2019; Wipf et al. 2019; Birnberg et al. 2020). Burkett-Cadena et al. (2016) showed that this approach has a potential to replace sentinel chickens for arbovirus surveillance programmes. Kurucz et al. (2014) also noted that this new system is capable of detecting of virus circulating in very low levels when no sentinel chickens seroconverted. Molecular detection of other vectors pathogens were also described for dried samples of ticks (Higgins et al. 2000). The identification of the vector insects blood meal dried on filter paper has been used in epidemiological studies of host feeding behaviour and infection status of vector population (Boid et al. 1999; Fall et al. 2012; Reeves et al. 2016; Niare et al. 2017).

DBS technology also provides valuable information for ecological surveys. A large-scale survey project DABSE based on collection and analysis of DBS was launched to monitor the impact of the most dangerous toxicants on wild bird species (Shlosberg et al. 2011). A common sampling technique for all types of birds makes it possible to assess their exposure to pollutants degree by the content of toxicants in the blood, regardless of their size, and without causing serious harm to them. For sampling on a card, 100–200  $\mu$ l of blood is enough, and the approach allows taking blood even from birds weighing 20 g. Following a unified methodology, researchers from all regions of the world can replenish the database of samples and collect statistics based on the results obtained. Under similar research the most dangerous factors affecting the biological activity and birds health caused by human use of pesticides, insecticides, antibiotics, heavy metals and other xenobiotics can be identified (Trudeau et al. 2007; Shlosberg et al. 2011, 2012; Lehner et al. 2013, 2018, 2020; Perkins and Basu 2018). Marine mammals are also at risk to be exposed to mercury (Hansen et al. 2014) and other environmental anthropogenic contaminants (Griffin et al. 2021), all these arise concern for potential adverse effects on wildlife populations. Thus,

McHuron et al. (2019) made a risk assessment of mercury concentration in hair, blood and DBS of marine wild-caught pinnipeds. In the Arctic, landlocked Arctic char was used for assessing mercury exposure as the sentinel fish (Barst et al. 2020). Bottlenose dolphins can be exposed to algal bloom toxins during red tides in coastal area, so these mammals are used as important sentinels to assess toxins exposure with the help of whole blood samples collected in the DBS form (Maucher et al. 2007; Twiner et al. 2011).

Dried biological material can be used for genetic studies, demonstrating the utility of membrane carrier (cards) for specimens collection, shipping, storage and further genetic data gathering. An important direction of DBS technology application is the causative agent sero-/genotyping and phylogenetic analysis (Michaud et al. 2007; Kennedy et al. 2008; Brito et al. 2008; Sintasath et al. 2009a, b; Madhanmohan et al. 2013; Bhuiyan et al. 2014; LeClaire et al. 2015; Jóźwiak et al. 2016; Ball et al. 2016; Manswr et al. 2018) as well as molecular detection/identification of the pathogen (Adams et al. 2006, 2008). Dried samples found application in populational genetics studies as a convenient instrument of sample collection and investigation (Sacks et al. 2004; Silva et al. 2009; Lall et al. 2010; Kraus et al. 2013; Guerini et al. 2014; Kashiwagi et al. 2015; Nunziata et al. 2016). Isolation and characterization of DNA microsatellite markers, their genotyping for population genetic studies was also described in other works (Sacks et al. 2004; King et al. 2005; Lucentini et al. 2006; Carr and Appleyard 2008; Heim et al. 2012; Nunziata et al. 2016). Kashiwagi et al. (2015) originally described an underwater collection of manta ray mucus by SCUBA divers for further application on FTA Elute cards for population genetic studies. An interesting application of the insect DNA extracted from dried samples, is insect identification for forensic entomology (fast and accurate estimation of time since death) (Harvey 2005). An investigations of dog leukocyte antigen extracted from DBS collected from dogs and wolves were successfully performed by Kennedy et al. (2008). Another example of such application – is an the amplification of endo- $\beta$ -1,4-glucanase gene from termites (Bujang et al. 2011) and first complete genomic sequence of a rabies virus obtained directly from FTA card (Goharriz et al. 2017). Borisenko et al. (2008) using DNA barcoding as a tool, performed verification of taxonomic identifications in ecological assessment surveys of small mammals (opossum, rodents, bats). Dried samples were also used for collecting, archiving and storage of mammals' and insects' genetic material (Smith and Burgoyne 2004; Owens and Szalanski 2005; Lall et al. 2010; Miller et al. 2013).

DBS samples are also useful for solving animal health important issues; for instance, measuring of fatty acids concentration as a valuable parameter in determining optimal dietary intake of nutrients in pigs (Wood et al. 2021a), health assessment of The San Cristóbal Galápagos tortoises (Dass



et al. 2021), African savanna elephants (Wood et al. 2021b), southern white rhinoceroses (Wood et al. 2021c) or assessment of wild-type diet composition and examination of fatty acid status for wild and managed care turtles (Dass et al. 2020; Koutsos et al. 2021). Wood et al. (2021b) showed that fatty acids profile in DBS, whole blood, serum and plasma of elephant were comparable. Vitamin D detection in DBS helps performing wildlife animals' health assessment (Higgins et al. 2020; Moittié et al. 2020), as well as captive animals' health evaluation (Michaels et al. 2015; Drake et al. 2017; Jaffe et al. 2019) as possible indication of nutritional metabolic bone disease (NMBD) due to possible vitamin D deficient diet. However, comparing vitamin D level in chimpanzees' DBS and serum, Moittié et al. (2020) concluded that these samples are not interchangeable and stressed that further studies on DBS evaluation are to be performed. Jaffe et al. (2019) also suggested that vitamin D3 detection in DBS is not yet a useful diagnostic method for corncrakes, due to significant systematic and random bias, although DBS and serum/plasma results were highly correlated. Both authors noted substantial analytical variability regardless of the sample type, and that should be connected with method itself (LC–MS/MS) along with sample pretreatment (sample elution and purification) rather than with poor applicability of DBS technology for vitamin D assay. DBS samples were also used to perform stable carbon isotope fingerprinting of essential amino acids as measures of Arctic char dietary carbon sources (Barst et al. 2020), as well as nitrogen stable isotope analyses of amino acids for determining trophic positions of the fish (Barst et al. 2021). Carbon and nitrogen stable isotopes measured in DBS can provide valuable information for feeding ecology or diet studies (O'Hara et al. 2018). Yee et al. (2013) used FTA cards to collect blood for DNA fingerprinting in order to identify painted and milky stork hybrids in zoo, combining both genetic and morphological data (plumage comparison). Dutra et al. (2020) assessed the welfare of dogs by measuring the relative telomere length, a biomarker of cellular ageing, in oral swabs and DBS. DBS can also be a source of DNA to identify sexes of wild birds, being especially important for ecology, biology and breeding (Gutiérrez-Corcheró et al. 2002; Quintana et al. 2009; Asawakarn et al. 2018).

### **DBS in veterinary and biology world: current trends and future developments**

In most publications covering the application of DBS technology, it is noted that the successful control of dangerous infections in livestock and wildlife requires an effective and inexpensive method for collection, shipping and storage of biological samples. In this matter, DBS technology is an important alternative to liquid or frozen biofluids, tissues

and other samples. However, in veterinary practice, the technology is not that popular, and is barely recognized in some countries; there are no domestic developments and approved guidelines for the use of dried samples in monitoring programs. The low demand for the technology in veterinary medicine is also associated with cost characteristics of the sampling cards available on the market, such as chemically treated FTA cards for PCR analysis, and convenience of its application to animal species, especially in the field. Mostly, the sampling devices are presented in the form of cellulose-based cards designed for dropwise application of biofluid. Typically, additional procedures and tools are required for blood sampling, which greatly complicates the process and increases the time spent per an individual. This format is of little use for whole blood sampling in wild nature, for these locations the stripes of absorbing material found wider application, such as Nobuto stripes, because of their utility for blood saturation from a puncture or a wound (Table 1). Generally speaking, for veterinary it would be preferable to use the easy-handling and affordable sampling devices that allow absorbing material saturation with blood and other biofluids without dispensers. Existing cellulose filter papers usually facilitate the function of a sample carriers with lack information on the amount of absorbed biomaterial and that complicates extraction procedure development. In this regard, alternative non-cellulose materials, such as fiberglass membrane, can provide volumetric microsampling of whole blood and other biofluids, which simplifies assay development (Samsonova et al. 2016, 2017, 2022).

DBS technology has its strengths and weak points, it is noted that there is lack of standardization of terminology and methodology (Smit et al 2014; Freeman et al. 2018). To provide accurate and reliable results for specific task, dried biosamples should be carefully evaluated vs liquid samples, taking into account analyte types and specimen, types of carrier, analyte extraction and assay conditions, samples storage peculiarities. Nevertheless, the review of the published works demonstrated that dried samples have been successfully used for a large variety of health issues across animal species, including birds, reptiles, amphibians, and other animals with small blood sample volumes. For wider adoption of DBS technology into veterinary practice, the convenient and affordable sampling devices should be supported with standardized procedures and available commercial reagents kits (such as ELISA and PCR), intended for the analysis of dried biological samples. This assists the routine use of DBS technology in veterinary laboratories, and provides an easy access for users from remote areas to animal health control.

Application of DBS technology for sampling, storage, transportation and analysis of biological samples of fauna species, has great potential and needs to be propagated not only in the area of agricultural production, but also for wild nature large-scale epidemiological investigations and survey programs. The combination of DBS and

serological analysis is a major advantage for large surveillance surveys. The control and diagnosis of dangerous diseases in livestock and wildlife, investigation of genetic diversity of causative agents and wildlife inhabitants, biobanking and ecological surveys are among the most important applications of DBS technology in veterinary medicine and biological investigations, since this technique meets the demands of easy collection and transportation of dozens of biological samples from herds and wildlife inhabitants to the laboratory. Animal specimens on filter paper are an economic, dry, non-hazardous way of biosamples shipping from collection site to the reference laboratory, making a good reason for the DBS technique to be widely adopted by veterinarians and biologist in their practice. This review is the first attempt to summarize the applications of DBS technology in veterinary and biology, and we hope that it will help to look deeper into the issue of some important technological and methodological points in the following publications concerning this area.

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