**PAPER** • **OPEN ACCESS**

# Glass-fiber membranes for storing, transportation and further characterization of agricultural plant biomaterial

To cite this article: I Vasyukova *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **433** 012011

# Glass-fiber membranes for storing, transportation and further characterization of agricultural plant biomaterial

**I Vasyukova[1], O Zakharova[1,2], Yu Altabaeva[1], S Kondakov[2,3], E Snegin[4], T Romanova[5] and A Gusev[1,2,6]**

[1]*G R Derzhavin Tambov State University*, 33 Internatsionalnaya Street, Tambov 392000, Russian Federation
[2]*National University of Science and Technology "MISIS",* 4 Leninsky Avenue, Moscow 119049, Russian Federation
[3]*Lomonosov Moscow State University*, 1-3 Lenin Hills, Moscow 119991, Russian Federation
[4]*Belgorod National Research University*, 85 Pobeda Street, Belgorod 308015, Russian Federation
[5]*Ogarev Mordovia State University*, 68 Bol'shevistskaya Street, Saransk 430005, Russian Federation
[6]*Voronezh State University of Forestry and Technologies named after G F Morozov*, 8, Timiryazeva Street, Voronezh 394087, Russian Federation

E-mail: nanosecurity@mail.ru

**Abstract**. The modified glass-fiber membranes possess high mechanical strength, good wetting and storage capacity, these properties render them a promising medium for storing dry biomaterial collected from agricultural plants. We have studied the applicability of this method for storing biomaterial collected from cucumber, tomato and potato in the form of dried spots on glass-fiber matrices for further ecological and phytosanitary studies. Also preservation of Phytophthora infestans deoxyribonucleic acid in the potato tuber and tomato fruit biomaterial stored on glass-fiber membranes has been evaluated. It has been revealed via real time polymerase chain reaction assays that in dried spots on glass-fiber membranes more than 90% of the plant deoxyribonucleic acid is preserved after seven-day storage. The method of electrophoretic isolation has shown that the condition of Phytophthora infestans deoxyribonucleic acid in the plant biomaterial stored on glass-fiber membranes is similar to the control after any storage period. In all the studied cases the storage conditions of the dried spots on glass-fiber matrices had no influence on the target deoxyribonucleic acid preservation. The obtained results prove usability of membrane carriers for crop science as a whole and detection of plant diseases in particular, and for food quality monitoring, especially carried out in the field.

## 1. Introduction

Animal and plant infectious diseases pose a serious challenge to the modern agriculture and crop science. According to the statistics, infectious diseases can reduce agricultural lands yields by 30-60%. Disease control is hampered by the lack of convenient and inexpensive methods for timely diagnostics even more than by the want for effective treatment methods. Conventional storage and transportation methods are extremely ill-suited for the field conditions as they require expensive refrigerating equipment as well as following a complicated research protocol.

The technique for storing genetic material in the form of dried spots was first suggested in 1963 by Guthrie and Susi [1] when blood samples were successfully stored on cellulose carriers for further metabolic diseases screening. Nowadays this method is referred to as “dried blood spot” (DBS) and is widely used for collecting and storing blood and other human biological fluids [2]. In recent years this technique has been introduced to veterinary medicine [3-5] and livestock husbandry [6].

Application of the dried spot technique for storage and transportation of plant biological materials with their further study by enzyme immunoassay (EIA), polymerase chain reaction (PCR) and other methods has also been proven viable in a number of research works. There is evidence that deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from tobacco leaves and from grape leaves and fruit can be successfully stored on cellulose cards for a long period at room temperature [7]. Chandrashekar et al. have shown that protein structures can be effectively preserved on commercially available cellulose cards treated with chemical agents for cell-lysing, denaturation of proteins and nucleic acids protection from nucleases, oxidation and UV-induced destruction (Whatman FTA cards), during their study PCR analysis was used to detect R. Solanacearum in biomaterial samples collected from infected tomato, potato, pepper and eggplants [8]. DNA-containing viruses can be detected in tissue prints on cellulose cards via PCR assay, as shown on the example of infected plants of tomato, corn, manioc, tobacco and on a number of woody plants. The authors emphasise that the results obtained from the samples stored on cellulose cards are absolutely equal to those obtained from conventionally-stored ones [9,10].

Development of glass-fiber matrices became a new stage in the technique of biomaterial sampling, storage and transportation as they have a number of advantages over previously used cellulose cards. The most important distinctive feature of glass-fiber material is inability of the sampled biological liquids to interact with the material and to penetrate individual fibers [11]. In addition, the modified glass-fiber membranes are characterized by high mechanical strength, good wettability and storage capacity, which makes them a preferable alternative both for researches and future consumers.

A number of recent research works have been aimed at revealing advantages and promising characteristics of glass-fiber membranes as media for biomaterial storage and transportation for further nanotoxicological testing [6,11] and medical studies [12-14]. Nevertheless, the question of applicability of the discussed material for crop cultivation has never received proper attention. Thus, our aim is to reveal the feasibility of application of glass-fiber membranes for storing plant biomaterial for further ecological and phytosanitary testing.

## 2. Materials and methods

### *2.1. Membrane medium characterization*

In our experiments plant biomaterial was stored and transported using cards with membrane carriers (membrane carrier, MC) Immunoved-NSP-1A (Immunoved, LLC, Moscow), designed for biological liquids sampling, transportation, storage and testing (figure 1).

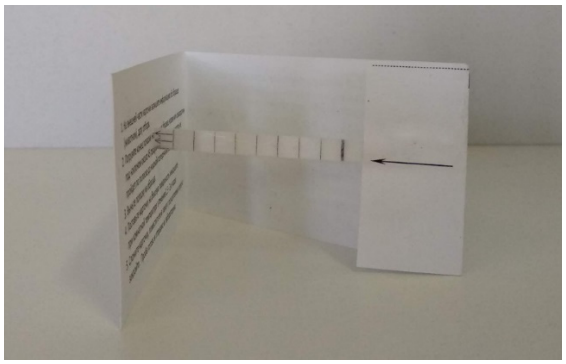
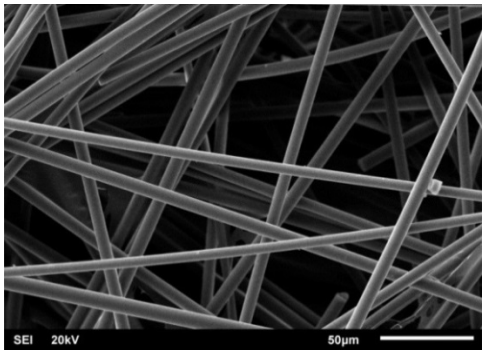


**Figure 1.** The layout of an Immunoved-NSP-1A membrane carrier card by Immunoved, LLC, Russia.

The working zone of the card consists of a strip of highly porous glass-fiber membrane composed of solid rod-like fibers. Figure 2 shows the material structure and main technical characteristics.




| Parameter | Value |
|---|---|
| Thickness | 0.35±0.05 mm |
| Specific density | 75 mg $cm^{-2}$ |
| Capillary rise | 35 mm $min^{-1}$ |
| Wetting time | 1.7±0.3 s $cm^{-1}$ |
| Specific adsorption capacity for water | 69±5 mg $cm^{-2}$ |
| Fiber diameter | 12±2 µm |

**Figure 2**. SEM micrograph and main technical characteristics of the working zone of an Immunoved-NSP-1A membrane carrier card.

*2.2. Biological samples characteristics*

For this study we selected several crops popular in central Russia: cucumber variety 'Zozulya', tomato variety 'Vityaz' and potato variety 'Gala'.

In the experiments with electrophoretic detection of PCR products tomato variety 'Vityaz' and potato variety 'Gala' infected with phytophthora rot (Phytophthora infestans (Mont.) de Bary) were tested.

*2.3. Biomaterial sampling using the membrane carrier*

Biomaterial for further testing was collected by submerging the working zone of an Immunoved-NSP-1A card into the sample (potato, tomato, cucumber) to a depth of 2-3 mm at 45°C angle until complete wetting of the working zone was achieved.

*2.4. DNA isolation*

The plant DNA for PCR assay was stored according to the following schemes:

1) Biomaterial in the native form, reaction completed.

2) Biomaterial applied to a test card, dried for 1 h at room temperature, rinsed from the card, reaction completed.

3) Biomaterial applied to a test card, dried for 1 h at room temperature, stored for 7 days at +42°C in a thermostat, rinsed from the card, reaction completed.

The following procedure was used for DNA isolation from the dried MCs transported to the laboratory: the working zone (5 x 5 mm) was cut off the card and submerged into 400 µl of lysis solution, the cell lysis was carried out with subsequent rinsing and DNA elution.

*2.5. Real-Time PCR*

Tomato, potato and cucumber samples were studied using the "Plant identification" test kit (SINTOL, Russia) designed for detection and identification of plant DNA in the process of food and food raw materials testing for vegetable-based GMO by the Real-Time PCR method (RT-PCR). The isolated DNA was tested using commercial reagent kits "DNK-Ekstran-1" for genomic DNA isolation (Syntol, Russia). As an intercalating dye, SYBR Green was used.

Real-Time PCR was carried out on an ABI StepOnePlus device (ThermoFisher Scientific, USA). PCR assays were performed in 20-35 cycles.

*2.6. PCR with electrophoretic detection*

In the experiments with electrophoretic detection of PCR products tomato variety 'Vityaz' and potato variety 'Gala' infected with phytophthora rot (Phytophthora infestans (Mont.) de Bary) were studied.

Electrophoretic detection was performed on an Amply 4 thermocycler (Biocom, Russia) for electrophoretic isolation of PCR products in agarose gel. The samples were tested for vegetable DNA and for Phytophthora infestans DNA using a reagent kit for analysis of PCR products by electrophoresis (AgroDiagnostica, LLC, Russia). The tests were carried out on native biological material and on 3-day and 7-day material samples stored on MCs.

To identify the DNA of potato and tomato, ready-made kits GM-104-50 "Potato" and GM-106-50 "Tomato" were used (Synthol, Russia). DNA amplification was performed according to the manufacturer's instructions.

To detect late blight, the following primers labeled SYBR Green: PiO8-3-3Fwd (5-CAATTCGCCACCTTCTTCGA-3), PiO8-3-3Rev (5-GCCTTCCTGCCCTCAAGAAC-3). Amplification Terms: Cycling conditions were 95 °C for 10 min followed by 40 cycles of 10 s at 95 °C, 15 s at 58 °C, 20 s at 72 °C, and fluorescence signal acquisition temperatures at 76 °C.

## 3. Results

*3.1. Evaluation of biomaterial preservation on MCs depending on the sample storage time*

Plant DNA preservation was evaluated both by means of RT-PCR and by analysis of PCR products by electrophoresis.

Below we present the results for the biomaterial preservation assessment.

In figure 3 one can see PCR kinetic curves for potato biomaterial in the native form and for biomaterial rinsed from membrane carriers after 1-hour and 7-day storage.

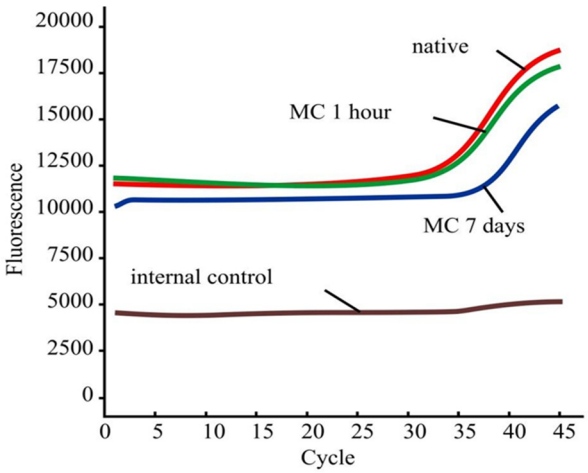



**Figure 3**. Kinetic curves: the PCR assay results for potato biomaterial samples.

According to the obtained results, potato DNA was detected in every sample, this proves that MCs are effective for storing potato genetic material. It is worth noting that the diagrams for the native and rinsed biomaterial samples are almost identical. Comparison of the MC-stored samples shows that in the 1-hour variant the maximal value for DNA fluorescence is about 17 500 units, while in the 7-day samples the fluorescence value is about 16 000 units. It follows, that DNA preservation after 7 days of storage is ~ 91%. The results for tomato are very similar.

According to the data (figure 4), tomato biomaterial stored on MCs yields the same results as freshly collected biomaterial.

Similarly to potato and tomato, preservation of cucumber DNA stored as dried spots on MCs is >90% (figure 5).

DNA preservation assessment by the fluorometric method showed that biological samples storage on membrane carriers is almost as effective as storage of the samples in the native form. It is safe to

assume that MCs can be successfully used for plant biomaterial storage for further ecological and phytosanitary testing by the methods of molecular diagnostics.

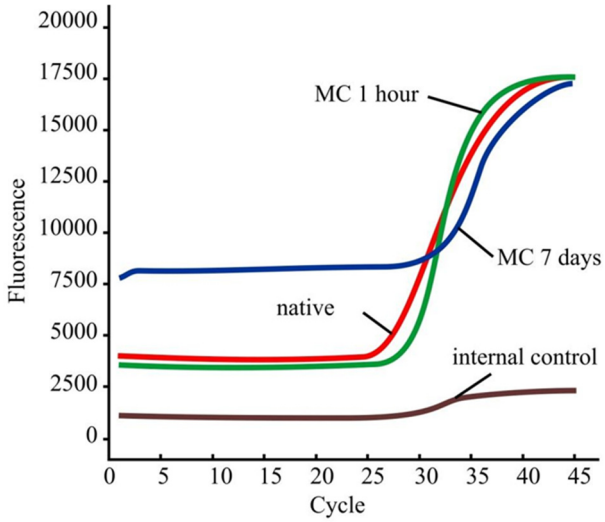



**Figure 4**. Kinetic curves: the PCR assay results for tomato biomaterial samples.

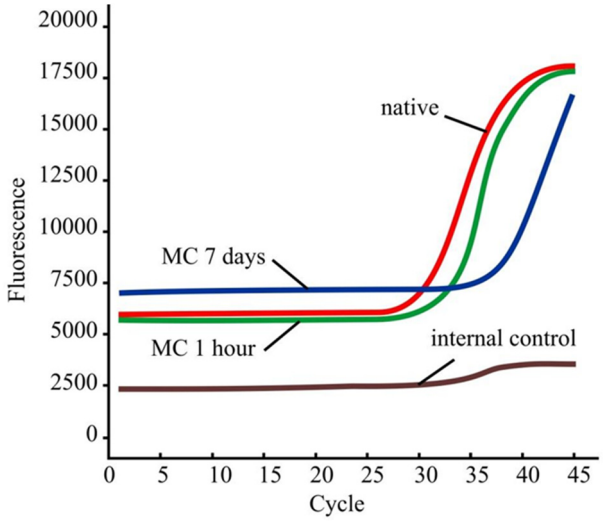



**Figure 5**. Kinetic curves: the PCR assay results for cucumber biomaterial samples.

*3.2. The effect of time on the safety of DNA of plants in MCs-stored*

Study of the results obtained through electrophoretic detection of PCR products of phytophthora DNA preservation in potato tubers and tomato fruit shows that the DNA is equally well preserved both in the native form (figures 6a, 7a) and in the dried spot on MCs (figures 6b-c, 7b-c). It should be noted that the storage time has no influence on the results, as the phytophthora DNA signal from a 7-day dried spot rinsed from a matrix carrier was clearly discernible (figures 6c and 7c).

The obtained results prove that membrane carriers can be successfully employed for plant diseases diagnostics and for quality monitoring in food industry.

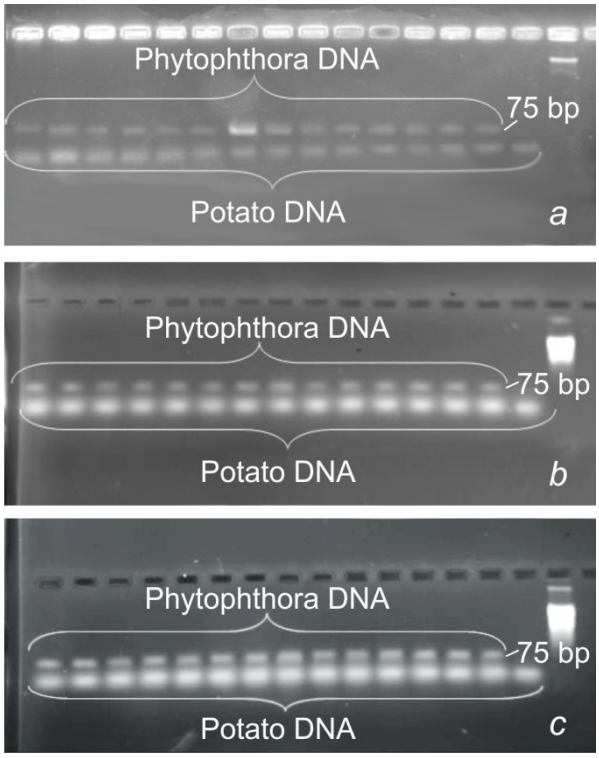



**Figure 6.** Results of PCR-assay with electrophoretic detection performed on potato. a) biomaterial in the native form; b) biomaterial on MCs after 1-hour storage; c) biomaterial on MCs after 7-day storage.

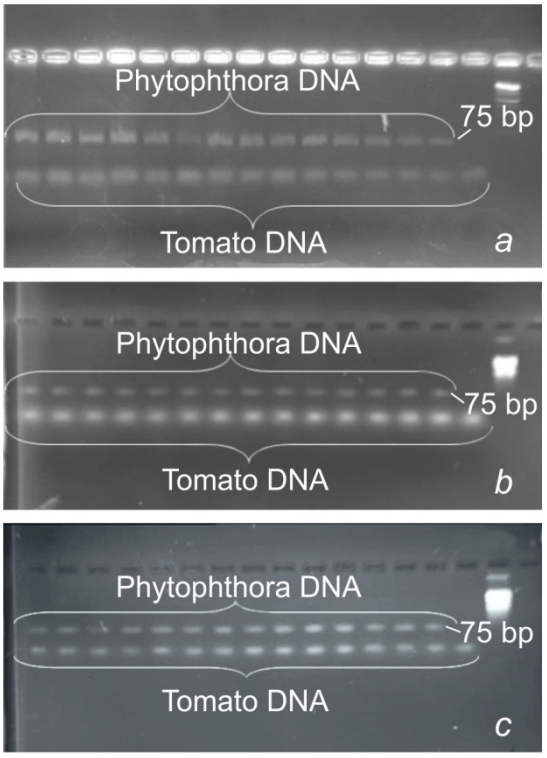



**Figure 7.** Results of PCR-assay with electrophoretic detection performed on tomato. a) biomaterial in the native form; b) biomaterial on MCs after 1-hour storage; c) biomaterial on MCs after 7-day storage.

## 4. Discussion

In today's world crop production diagnostic procedures are usually carried out on whole biological fluids. Firstly, such analytical objects often require freezing with rigorous adherence to the temperature regime, which is inconvenient or impossible under field conditions; secondly, in order to obtain reliable results one requires a large bulk of biological material, especially if tests are performed in multiple replications; thirdly, storage and transportation of large masses of frozen samples is always very costly.

The results of our research prove that the dried spot technique is a good alternative to conventional approaches to biomaterial sampling, storage and transportation for further molecular diagnostics. This method ensures good preservation of genetic material and provides consistent results as proven by PCR assays performed on a variety of crops.

The ecological situation in the modern world together with the human-induced impact on the environment result in massive contamination of crops with a wide range of toxic agents, including heavy metals. Matrices for dry storage of biological material at room temperature can facilitate collection and transportation of biological samples for regular monitoring levels of toxic agents, for example, heavy metals, in plants. Such studies have already been performed for livestock products [15].

Beside inorganic pollutants, plant cultivation suffers from the spread of virus-induced diseases where late detection leads to reduction in yields and to general financial losses. The described dried spot method proves effective for plant biomaterial storage for further detection of foreign DNA. [7,8,16].

A number of research works have shown that RNA and DNA from plants and phytopathogens can be stored as dried spots on cellulose carriers [7,9,17]. However, MCs can be more efficient than cellulose-based carriers for the applications connected with plant cultivation because of their inertness towards biological components, and because liquids do not penetrate individual glass fibers. While high

degree of preservation of the most important sample characteristics renders this method useful for phytopathology detection in crop production and for food quality monitoring.

Fast growth of genetic engineering has led to increased demand for monitoring the amount of genetically modified products in agriculture and in food industry. The dried spot storage method for biological samples could be of great use for researchers as its applicability in combination with PCR-analysis has been proven experimentally [7,18].

Determination of antibiotic and hormon residues in food could be another highly promising application for the dried spot storage method for biological samples. The accumulated experimental and theoretical data on the foods of animal origin evaluation [19-21] allow one to assume that in the nearest future this approach will become the main method for biosamples storage and transportation for further screening.

The technology for storing vegetable biomaterial, beside being undoubtedly useful for agricultural applications, has a big potential for the wild plants genome biobanking [22].

## 5. Conclusion

Timely diagnosis of diseases of agricultural plants using modern high-precision molecular methods can solve a number of problems in the field of crop production. However, their widespread adoption is hindered by the high cost, complex sample preparation and compliance with strict transportation rules, as well as the need to carry out diagnostics in the laboratory. At least one of the identified problems can be solved with the help of MCs. The results of our studies show that the use of MCs application as media for storage, transportation and subsequent testing of plant-based biosamples is an effective, economically viable and sensitive method both for laboratory and field conditions. The preservation of plant and phytopathogenic DNA samples at the level of 90% or more allows reliable results to be obtained in subsequent molecular diagnostics. Introduction of this technology into practice can considerably widen the potential for use of modern molecular methods for diagnostics of phytopathogens of agricultural crops. However, to obtain confirmation of the applicability of this method, it is necessary to assess the degree of preservation of the biological material of plants and phytopathogens in a wider species range.

## 6. Acknowledgments

The project was partially supported by the Russian Fund for Basic Research (Project No 17-44-680001).

## References

[1] Guthrie R, Susi A A 1963 Simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* **32** 338

[2] Lee S B et al 2011 Assessing a novel room temperature DNA storage medium for forensic biological samples. *Forensic Science International: Genetics* **6**(1) 31 https://doi.org/10.1016/j.fsigen.2011.01.008

[3] Rosypal A C, Pick L D, Hernandez J O 2014 Evaluation of a novel dried blood spot collection device (HemaSpot™) to test blood samples collected from dogs for antibodies to Leishmania infantum. *Veterinary Parasitology* **205(**1–2) 338 https://doi.org/10.1016/j.vetpar.2014.07.031

[4] Andersson A M, Reineck H B, Nyman A K, Wallgren P 2015 Quantitative detection of antibodies to aleutian disease virus in dried blood spots as an estimation of hypergammaglobulinemia in mink. *Virol-mycol* **4** 7 https://doi.org/10.4172/2161-0517.1000147

[5] Saushkin N Y, Samsonova J V, Osipov A P, Kondakov S E, Lysova E S, Elizarova I A, Khaertynov K S, Shuralev E A 2018 Strip-Dried biofluids for the detection of specific antibodies in infected small ruminants. *Moscow University Chemistry Bulletin* **73(**3) 135 https://doi.org/10.3103/S0027131418030069

[6] Samsonova J V, Chadina A S, Osipov A P, Kondakov S E, Makarova T E, Komarov A B 2014 Detection of bovine leukemia virus by the polymerase chain reaction in dried blood spots using a membrane system of a new format. *Moscow University Chemistry Bulletin* **69(**6) 282

https://doi.org/10.3103/S0027131414060066
[7] Roy Y, Nassuth A 2005 Detection of plant genes, gene expression and viral RNA from tissue prints on FTA cards. *Plant Molecular Biology Reporter* **23**(4) 383 https://doi.org/10.1007/BF02788886
[8] Chandrashekara K N, Prasannakumar M K, Deepa M, Vani A 2012 A rapid, sensitive and reliable method for detecting Ralstonia solanacearum using FTA (whatman) card. *Journal of Plant Pathology* **94(**1) 219 http://dx.doi.org/10.4454/jpp.fa.2012.012
[9] Ndunguru J, Taylor N J, Yadav J, Aly H, Legg J P, Aveling T, Thompson G, Fauquet C M 2005 Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. *Virology Journal* **2** 45 https://doi.org/10.1186/1743-422X-2-45
[10] Janos Á, Borjana S, Kalin D, Istvan T, Laszlo P 2015 Use of FTA (Whatman) membrane for collection and detection of Bulgarian PPV isolates. *Journal of Mountain Agriculture on the Balkans* **18(**2) 334
[11] Metelkin A, Kuznetsov D, Kolesnikov E, Chuprunov K, Kondakov S, Osipov A, Samsonova J 2015 Membrane materials for storing biological samples intended for comparative nanotoxicological testing. *IOP Conf. Series: Materials Science and Engineering* **98** 012019 https://doi.org/10.1088/1757-899X/98/1/012019
[12] Corran P H et al 2008 Dried blood spots as a source of anti-malarial antibodies for epidemiological studies. *Malar J* **7** 195 https://doi.org/10.1186/1475-2875-7-195
[13] D'Avolio A, Simiele M, Siccardi M, Baietto L, Sciandra M, Bonora S, Di Perri G 2010 HPLC–MS method for the quantification of nine anti-HIV drugs from dry plasma spot on glass filter and their long term stability in different conditions. *Journal of Pharmaceutical and Biomedical Analysis* **52(**5) 774 https://doi.org/10.1016/j.jpba.2010.02.026
[14] Lawson G, Cocks E, Tanna S 2012 Quantitative determination of atenolol in dried blood spot samples by LC–HRMS: A potential method for assessing medication adherence. *Journal of Chromatography B* **897** 72 https://doi.org/10.1016/j.jchromb.2012.04.013
[15] Lehner A F, Rumbeiha W, Shlosberg A, Stuart K, Johnson M, Domenech R, Langner H 2013 Diagnostic analysis of veterinary dried blood spots for toxic heavy metals exposure. *Journal of Analytical Toxicology* **37** 406 https://doi.org/10.1093/jat/bkt048
[16] Nagata T, Inoue-Nagata A K, de Ávila A C, de B Giordano L 2004 Print-capture PCR for detection of tomato begomoviruses from plants and whiteflies. *Fitopatol bras* **29(**1) 91 http://dx.doi.org/10.1590/S0100-41582004000100014
[17] Burlakoti R R, Hsu C-f, Chen J-r, Sheu Z-M, Bihon W, Kenyon L 2019 Capture of Ralstonia solanacearum species complex strains directly from plant tissue sampled on FTA cards for molecular characterization. *J. of Plant Pathology* doi: 10.1007/s42161-019-00361-z
[18] Lin J J, Fleming R, Kuo J, Matthews B F, Saunders J A 2000 Detection of plant genes using a rapid, nonorganic DNA purification method. *Biotechniques* **28(**2) 346 https://doi.org/10.2144/00282pf01
[19] Wilhelm A J, den Burger J C G, Swart E L 2014 Therapeutic drug monitoring by dried blood spot: progress to date and future directions. *Clin. Pharmacokinet* **53** 961 https://doi.org/10.1007/s40262-014-0177-7
[20] Sharma A, Jaiswal S, Shukla M, Lal J 2014 Dried blood spots: Concepts, present status, and future perspectives in bioanalysis. *Drug Testing and Analysis* **6(**5) 399 https://doi.org/10.1002/dta.1646
[21] Gaissmaier T, Siebenhaar M, Todorova V, Hüllen V, Hopf C 2016 Therapeutic drug monitoring in dried blood spots using liquid microjunction surface sampling and high resolution mass spectrometry. *Analyst* **141** 892 https://doi.org/10.1039/c5an02302e
[22] Siegel C S, Stevenson F O, Zimmer E A 2017 Evaluation and comparison of FTA card and CTAB DNA extraction methods for non-agricultural taxa. *Applications in Plant Sciences* **5(**2) 1600109 https://doi.org/10.3732/apps.1600109