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Collection of plasma samples in areas with limited healthcare access

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Abstract

Regular monitoring of various biomarkers and molecular panels in plasma can significantly help to prevent disease onset and improve its management and final outcomes. Many groups can benefit from monitoring programs focusing on the prevention of cardiovascular diseases, evaluation of environmental exposure impacts, or the prevention/management of cancer. Improvement in therapeutic options in part due to targeted therapeutic agents and monoclonal antibody therapies has led to a significant sized population that can be described as “cancer survivors”. These patients, although in remission from their original disease, are at significant risk for the recurring disease and must be monitored for adverse events. Monitoring is, however, not an easy task; requiring a high level of complexity in lab facilities and blood/plasma sampling, collection and storage must occur under tightly controlled conditions. These demanding circumstances are especially difficult to attain in rural areas and in historically marginalized populations. The Telimmune Plasma Separation Card (TPS card or TPSC) has been developed to enable diagnostic plasma sampling, collection and stabilization in locations that may be remote to laboratory or clinic. The TPSC requires a drop of blood applied to a top of a separation system consisting of a separation membrane and collection disk. In three minutes, the TPSC device separates plasma from erythrocytes and deposits a defined volume of plasma into a collection disc which is air dried for 15 minutes to deliver a stabilized, volumetric plasma sample, which may be stored or shipped at ambient temperatures with minimal biological risk. Extraction of proteins and metabolites is then achieved in well-equipped laboratories using protocols discussed in this chapter.

1. Introduction

Most clinical assays begin with blood collection in a clinical setting, requiring a phlebotomist to draw blood and a laboratory to prepare serum or plasma for analysis. Difficulties associated with collection, stabilization, storage and shipping of plasma samples, from rural/remote areas to certified laboratories in urban areas can result in the under-diagnosis of many diseases and/or inefficient monitoring of patients. Several plasma separation devices have been developed in the last few decades. These platforms are generally based on filtration [1-5], centrifugation strategies [6,7], diffusion methods [8,9], Zweifach-Fung Effect [10,11] and physical removal of cells [12]. Although these are promising technologies, they require either trained person to operate them or an energy source. Samples can be also collected as Dried blood spot (DBS) using simple filter paper. Results from such samples are, however, difficult to standardized due to the highly variable hematocrit level [13-18] ranging between 20-70% and may change significantly during a day [19-21]. These obstacles often prevent systematic and high-frequency collection of valuable biomarker data from patients. The recently developed Telimmune Plasma Separation Card (TPS card or TPSC) [22] is a device designed to collect a specific volume of the plasma independent of blood hematocrit and viscosity and is therefore ideal for biomarker profiling studies and biomarker quantification. The TPSC requires no power and circumvents requirements for venipuncture-phlebotomy training, needles, special collection vials, refrigeration, and centrifugation generally associated with traditional plasma methods and therefore is ideal for plasma sample collection in dispersed locations or by patients directly at home. Plasma samples collected using the TPSC are very stable [23-25], do not require refrigeration or other special conditions and may be safely conveyed to the clinical laboratory by regular mail.

Reproducibility of volume sampling was examined based on data from ten extraction card experiments using blood from the same individual [22]. The impact of hematocrit levels

of 20%, 41%, and 71% on sample aliquot volume were tested [22]. Inter-card variation in aliquots was <1% for the 20% and 41% hematocrit samples while that for the 71% sample was 3.4% [22]. The % RSD for the three different hematocrit levels was <1%. Moreover, with the 41% hematocrit level the % RSD of volume difference between venous and finger-stick blood was negligible <2% [22]. This demonstrates that the TPSC is capable of reproducible sample collection regardless of hematocrit or volume of the blood applied. Metabolite and protein biomarkers recovery from the TPSC were also extensively evaluated for both protein and metabolite-based analysis. For example, dried samples were extracted from the disc and analyzed for 25-Hydroxy Vitamin D by LC-MS/MS analysis using derivatization with a secosteroid signal enhancing tag [22].

The TPSC was tested in two studies of *Helicobacter pylori* exposer diagnosis in both US [26] and developing countries [27]. In both cases, plasma was collected using of TPSC and dried collection disks were analyzed by conventional EIA test in a laboratory setting. Kearney et al. [26] tested 84 patients for the presence of antibodies to *H. pylori* in blood samples obtained by venipuncture and by the TPSC. There was a high degree of correlation of quantitative EIA results between specimens obtained by venipuncture as compared to those obtained by the TPSC (r=0.98) [26]. There was also a high degree of correlation between duplicated cards (r=0.996) [26]. The qualitative results from the TPSC and venipuncture specimen testing differed in 7 of 84 patients [26]. The sensitivity and specificity of the TPSC were calculated using the venipuncture specimen as the gold standard [26]. This revealed a sensitivity of 93 % and a specificity of 100 % in the first duplicate and 98% in the second duplicate [26]. In the second study, Nurgalieva et al. analyzed a total of 289 simultaneously obtained plasma (using TPSC) and serum (using venipuncture) samples from individuals in Kazakhstan [27]. Plasma samples were air-dried for 15 min, stored desiccated in foil zip-lock pouches at 4 to 6°C, and subsequently shipped to the US by air at ambient temperature [27].

Serum samples remained frozen at -20°C until assayed. *H. pylori* status was determined by enzyme-linked immunosorbent assay [27]. The results were concordant in 250 of the 289 cases (86.5%) [27]. In 25 cases (8.6%), the dry plasma samples gave indeterminate results and could not be retested because only one sample was collected [27]. Five serum samples were positive, and the corresponding dry plasma samples were negative; one serum sample was negative, and the corresponding plasma sample was positive [27]. The relative sensitivity and specificity of the TPSC samples to serum were 97.6 and 97.9%, respectively, excluding those with indeterminate results [27]. Repeated freeze-thawing had no adverse effect on the accuracy of the test (coefficient of variation of <6%) and reproducibility was determined at 6% [27].

Recently, the TPS cards were used and validated for both protein [23] as well as metabolite [24] targets indicating high sample stability and assay reproducibility, and further demonstrating the potential to enable easy access to biomarker assays. In this chapter, detailed protocols for protein metabolite extraction from the TPS Cards are described with a focus on discovery-based approaches for analyses of intact proteins, peptide-based proteomics, and GC- and LC-MS-based metabolomics.

2. Materials

2.1. Plasma collection and storage

1. Telimmune Plasma Separation Card (TPSC) (Telimmune, West Lafayette, IN, USA) (see Note 1).
2. Lancet: McKesson Safety Lancets, Sterile, Push-Button, 17 Gauge Blade (McKesson, Irving, TX, USA) (see Note 2).
3. Alcohol Prep Pads: CareTouch, individually wrapped alcohol prep pads (CareTouch, Brooklyn, NY, USA).

2.2. Extraction of intact proteins – General procedure

1. TPSC Extraction Buffer: 50 mM Tris-HCl (pH 8) (Sigma-Aldrich, Burlington, MA, USA), and 150 mM NaCl (Sigma-Aldrich, Burlington, MA, USA) (see Note 3).
2. Corning™ 96-Well Nonbinding Surface (NBS™) Microplates with Lid (Fisher Scientific, Waltham, MA, USA).
3. Electron Microscopy Sciences Branson Ultra Sonic Bath Model 5800-MTH (Fisher Scientific, Waltham, MA, USA) (see Note 4).

2.3. Extraction of intact proteins – SARS-CoV-2 total Ab assay

1. TPSC Extraction Buffer: 50 mM Tris-HCl (pH 8) (Sigma-Aldrich, Burlington, MA, USA), and 150 mM NaCl (Sigma-Aldrich, Burlington, MA, USA).
2. Corning™ 96-Well Nonbinding Surface (NBS™) Microplates with Lid (Fisher Scientific, Waltham, MA, USA).
3. Electron Microscopy Sciences Branson Ultra Sonic Bath Model 5800-MTH (Fisher Scientific, Waltham, MA, USA) (see Note 4).
4. Fluorescence Reader BioTek Synergy 2 (BioTek, Winooski, VT, USA)

5. Platelia SARS-CoV-2 Total Ab Assay (Bio-Rad, Hercules, CA, USA) including:
    a. R1 Microplate: 12 strips of 8-wells each, coated with recombinant nucleocapsid protein of SARS.
    b. R7 Sample Diluent: TRIS-NaCl buffer, Phenol red, ProClin 300 (0.5%).
    c. R8 TMB Substrate Buffer: Hydrogen peroxide, Citric Acid/Sodium Acetate buffer, Dimethylsulfoxide (DMSO).
    d. R9 Chromogen: Solution containing Tetramethylbenzidine (TMB).
    e. R10 Stopping Solution: 1N Sulfuric acid solution (H_2SO_4).

2.4. Extraction and digestion of proteins for peptide-based LC-MS proteomics – Direct digestion

1. TPSC Denaturation Buffer: 50 mM ammonium bicarbonate, pH 8.0 (Sigma-Aldrich, Burlington, MA, USA); 10 mM TCEP (Sigma-Aldrich, Burlington, MA, USA); 5% SDC (sodium deoxycholate) (Sigma-Aldrich, Burlington, MA, USA). Aliquots can be stored at -80°C.
2. TPSC Alkylation Buffer: 100 mM Iodoacetamide (Sigma-Aldrich, Burlington, MA, USA). Aliquots can be stored at -80°C.
3. TPSC Dilution Buffer: 50 mM Ammonium Bicarbonate, pH 8.0 (Sigma-Aldrich, Burlington, MA, USA).
4. TPSC Trypsin Solution: Prepare the Trypsin (MS grade; Sigma-Aldrich, Burlington, MA, USA) solution in a concentration of 1 µg/µL using an appropriate volume of TPSC Dilution Buffer.

2.5. Extraction and digestion of proteins for peptide-based LC-MS proteomics – SP3 digestion

1. TPSCSP3 Denaturation Buffer: 50 mM ammonium bicarbonate, pH 8.0 (Sigma-Aldrich, Burlington, MA, USA); 1% SDS (Sigma-Aldrich, Burlington, MA, USA); 1% Triton X-100 (Sigma-Aldrich, Burlington, MA, USA); 1% NP40 (Sigma-Aldrich, Burlington, MA, USA); 1% Tween 20 (Sigma-Aldrich, Burlington, MA, USA); 1% SDC (Sigma-Aldrich, Burlington, MA, USA); 5 mM EDTA (Sigma-Aldrich, Burlington, MA, USA); 50 mM NaCl (Sigma-Aldrich, Burlington, MA, USA); 1% Glycerol (Sigma-Aldrich, Burlington, MA, USA;, 10 mM TCEP (Sigma-Aldrich, Burlington, MA, USA).
2. TPSC Alkylation Buffer: 100 mM Iodoacetamide (Sigma-Aldrich, Burlington, MA, USA). Aliquots can be stored at -80°C.
3. TPSC Dilution Buffer: 50 mM Ammonium Bicarbonate, pH 8.0 (Sigma-Aldrich, Burlington, MA, USA).
4. SP3 Activated Beads (for 10 samples): Sera-Mag E3 and E7 beads are provided in NaN3 solution at concentration 50 mg/mL each. The following procedure will generate a solution of activated beads at a concentration of 50 µg/µL each with a final volume of 110 µL. This is good for 10 samples (10 µL/sample) plus 10% (10 µL) as a backup.
    a. Mix well provided bottles.
    b. Mix 55 µL E3 and 55 µL E7 suspensions in a new Eppendorf tube.
    c. Place the tube in a magnetic rack until all beads moved to the tube wall.
    d. Carefully discard supernatant without disturbing fixed beads and discard supernatant (~80 µL).
    e. Remove the tube from the magnetic rack, add 180 µL of water and mix gently by pipetting.
    f. Place the tube in a magnetic rack until all beads moved to the tube wall.
    g. Carefully discard supernatant without disturbing fixed beads.

h. Remove the tube from the magnetic rack.

i. Add 80 µL water and mix gently by pipetting.

5. TPSC Trypsin Solution: Prepare the Trypsin (MS grade; Sigma-Aldrich, Burlington, MA, USA) solution in a concentration of 1 µg/µL using an appropriate volume of TPSC Dilution Buffer.

2.6 Extraction of metabolites for global profiling

1. Methanol (MS grade; Fisher Scientific, Waltham, MA, USA).
2. Chloroform (HPLC grade; Fisher Scientific, Waltham, MA, USA).
3. Ultrapure, filtered water.
4. Ribitol (Adonitol) (Sigma-Aldrich, Burlington, MA, USA).
5. N-methyl-N-trimetylsilyltrifluoroacetamide (MSTFA) (MS grade; Sigma-Aldrich, Burlington, MA, USA).
6. Pyridine, 99.5%, Extra Dry over Molecular Sieve, AcroSeal (Fisher Scientific, Waltham, MA, USA).
7. Fatty acid methyl ester (FAMEs) standard mixture (Sigma-Aldrich, Burlington, MA, USA) (see Note 5).

3. Methods

Telimmune Plasma Separation Card collects 3 µL of plasma aliquot per collection disk (one disk in UNO format, two disks in DUO format) (Figure 1). For DUO cards, a minimum volume of 45 µL is needed to fill the test spot. For UNO, a minimum volume of 25 µL is needed to fill the test spot (see Note 6). Assuming protein concentration in plasma is in a range of 60-80 mg/mL, each disk with 3 µL collected plasma will contain ~180 - 240 mg

of proteins (primarily represented by high abundant proteins including Albumin, Immunoglobulins, Transferrin, etc.). Typical recovery of proteins from the disk is 80-100% [23]. Similar recovery applies to metabolites studied [24]. Recently, TPSC was also used to determine presence of SARS-CoV-2 antibodies in plasma samples. These experiments demonstrate a high degree of correlation between cards and liquid samples (r=0.977) (Figure 2, adapted from Telimmune).

3.1. Plasma collection and storage

1. Open the card package where indicated. Save the sleeve to place the card back in after the procedure is over (see Note 7).
2. Clean the area to be punctured using an alcohol wipe. Make sure your hands are warm (see Note 8).
3. Press the lancet firmly against your skin then release the lancet by pressing the top button (see Note 9).
4. Apply pressure at the base of your finger towards the tip to increase the size of the blood drop. Keep the drop pointed towards the card (hand turned vertical) to keep the blood from spreading on your finger.
5. Deposit two large hanging drops of blood to the indicated surface port. Do not touch the surface.
6. Allow 30 seconds after initial blood application to make sure the indicated “control spot” is filled. If it doesn’t fill, apply another drop (see Note 10).
7. Peel to remove the top layer of card at 3 minutes.
8. Air-dry collection disk for 15 minutes at room (ambient) temperature (see Note 11).

9. Fold the TPS Card in the middle with the collection disc inside the fold, then place back into the sleeve. The card can be stored at ambient temperature for several days (weeks) or shipped/transported to a clinical laboratory (see Note 12).

3.2. Extraction of intact proteins – General procedure

1. Place a disk into a well of 96 well plate with a flat bottom (see Note 13).
2. Add 50 µL TPSC Extraction Buffer. Make sure the disk is completely covered.
3. Incubate for 1 hour at room temperature.
4. Add another 50 µL TPSC Extraction Buffer, mix well with a micropipette and transfer the entire volume (~100 µL) to a new well or Eppendorf tube (see Note 14).
5. Continue with the recommended assay/kit protocol used to analyze the sample.

3.3. Extraction of intact proteins – SARS-CoV-2 total Ab assay

1. Place a disk into a well of 96 well plate with flat bottom.
2. Add 10 µL TPSC Extraction Buffer and 40 µL R7. Make sure the disk is completely covered.
3. Incubate for 1 hour at room temperature.
4. Add 50 µL R6, mix well with a pipette and transfer the entire volume (~100 µL) to the well of the reaction microplate R1 (see Note 15).
5. Cover the microplate with an adhesive plate sealer or use other means to minimize evaporation. Incubate the microplate in a controlled 37°C +/- 2°C water bath or microplate incubator for 60 minutes (+/- 5 min).
6. Prepare the enzyme development solution with a 1:11 dilution of the Chromogen (R9) in the Substrate Buffer (R8). Mix thoroughly.
7. At the end of the incubation period, carefully remove the plate cover. Aspirate the contents of all wells into a biohazard waste container (containing sodium

hypochloride). Wash the plate 5 times with a microplate washer (using 800 µL of Working Washing Solution per well). Invert the microplate and gently tap on absorbent paper to remove the remaining liquid.

8. Quickly add 200 µL of the development solution (R8+R9) into each well. Incubate plates in the dark for 30 minutes at room temperature. Do not use an adhesive plate sealer during this incubation step.
9. Add 100 µL of Stopping Solution (R10) to each well, using the same sequence and rate of addition as for the development solution. Mix thoroughly.
10. Carefully wipe the plate bottom.
11. Read the optical density of each well at 450 nm (reference filter at 620 nm) within 30 minutes after the addition of the Stopping Solution. The strips must be protected from light before reading.

3.4. Extraction and digestion of proteins for peptide-based LC-MS proteomics – Direct digestion

1. Place a disk into a well of 96 well plate with a flat bottom (~200 µg proteins).
2. Add 20 µL TPSC-S Denaturation Buffer - make sure the disk is fully covered with the buffer.
3. Incubate for 10 min at 60°C (top of the well in the plate must be covered to prevent evaporation).
4. Add 5 µL TPSC Alkylation Buffer and incubate an additional 60 mins at room temperature in dark (top of the well in the plate must be covered to prevent evaporation).
5. Dilute sample with 170 µL TPSC Dilution Buffer, mix well.
6. Add 2 µL TPSC Trypsin Solution.
7. Incubate overnight at 37°C.

8. Add 10 µL of 20% FA and incubate for 30 min at room temperature.
9. Centrifuge at 15,000 x*g*.
10. Transfer 150 µL of supernatant into the new tube - sample is ready for analysis (at concentration ~ 1 µg/µL).

3.5. Extraction and digestion of proteins for peptide-based LC-MS proteomics – SP3 digestion (see Note 16)

1. Place a disk into a well of the 96 well plate with a flat bottom (~200 µg proteins).
2. Add 192 µL TPSCSP3 Denaturation Buffer.
3. Incubate for 10 min at 60°C (tube must be closed to prevent evaporation).
4. Add 5 µL TPSC Alkylation Buffer (~1 µg/ µL final protein concentration) and incubate an additional 60 min at room temperature in dark (top of the well in the plate must be covered to prevent evaporation).
5. Transfer 25 µL of alkylated sample from the previous step to the new Eppendorf tube and mix it with 65 µL TPSCSP3 Denaturation Buffer.
6. Add 10 µL SP3 Activated Beads (50 µg/µL; 500 µg beads total) and mix gently by pipetting.
7. Add 100 µL EtOH (100%) and mix gently by pipetting.
8. Incubate the mixture at RT for 5 min – mix slowly with < 1,000 rpm.
9. Place tubes in a magnetic rack until all beads moved to the tube wall.
10. Carefully discard supernatant without disturbing fixed beads.
11. Remove the tubes from the magnetic rack, add 180 µL of 80% EtOH and mix gently by pipetting.
12. Repeat steps 9-11 two more times.
13. Place tubes in a magnetic rack until all beads moved to the tube wall.

14. Carefully discard supernatant without disturbing fixed beads, make sure there is no residual EtOH solution (< 5 µL).
15. Remove the tubes from the magnetic rack, add 98 µL TPSC Dilution Buffer, 2 µL TPSC Trypsin Solution.
16. Sonicate for 30 seconds in a water bath to fully disintegrate the beads.
17. Incubate for 1 hour at 37°C; after that, mix gently by pipetting.
18. Incubate overnight at 37°C.
19. Place tubes in a magnetic rack until all beads moved to the tube wall.
20. Carefully transfer supernatant to the new tube. Make sure not to disturb the fixed beads.
21. Centrifuge at 15,000 x*g* to remove any remaining beads.
22. Transfer 80 µL supernatant into the new tube.
23. Dry in speed vac (do not over dry).
24. Reconstitute a pellet in 20 µL 0.01% FA. The sample is ready for analysis at concentration ~ 1 µg/µL.

3.6. Extraction of metabolites for global profiling (see Note 17)

1. Place dried disc into a 1.5 mL Eppendorf tube.
2. Add 500 µL of 100% MeOH, vortex briefly on highest setting.
3. Add 30 µL of Ribitol (0.2 mg/mL stock in water) – this serves as an internal standard for GC-MS analysis.
4. Incubate in 70°C dry bath for 15 minutes (after 2-3 minutes open Eppendorf tubes to release built-up pressure to prevent tubes from bursting open and loss of sample).
5. Centrifuge for 10 minutes at 18,400 xg to remove any remaining debris.
6. Transfer supernatant to a clean Eppendorf tube.
7. Add 250 µL of $CHCl_3$.

8. Add 500 µL of ultra-pure, filtered water and vortex for 15 seconds on highest setting.
9. Centrifuge for 15 minutes at 1,500 xg to further separate phases.
    a. Transfer 700 µL of the upper polar phase (or two aliquots of 350 µL) to a clean Eppendorf tube. Dry using a speed vacuum at room temperature and prepare samples for GC-MS analysis (see Note 18).
    b. Add 40 µL Methoxyaminehydrochloride (prepared to a concentration of 20 mg/mL in pyridine).
    c. Shake at 37°C, 950 rpm for 2 hours.
    d. Shortly spin down to remove evaporated drops on Eppendorf lid.
    e. Add 70 µL MSTFA mixture (1 mL of MSTFA + 20 µL of FAME mixture).
    f. Shake at 37°C, 370 rpm for 30 minutes.
    g. Centrifuge at maximum speed to remove any precipitate solution.
    h. Transfer 90 µL of the now derivatized sample into a clear glass vial with an insert.
    i. Transfer another 10 µL of each sample to another vial to create a quality control pool. Samples are now ready for GC-MS analysis.
10. Transfer 200 µL of the lower nonpolar phase to a clean glass vial. Dry using a constant stream of nitrogen (N_2) gas at room temperature and prepare samples for LC-MS analysis (see Note 18).
    a. Add 48 µL of 100% methanol and 2 µL of EquiSPLASH Lipidomix Standard Mixture to serve as an internal standard to each glass vial.
    b. Vortex gently or carefully pipette up and down to ensure proper resuspension of sample.
    c. Transfer to clean Eppendorf tube, centrifuge at maximum speed to remove any precipitate from solution.

d. Transfer 45 µL to a clear glass vial with an insert.

e. Transfer another 5 µL of each sample to another vial to create a quality control pool. Samples are now ready for LC-MS analysis.

4. Notes

1. TPS Cards are available in two formats, UNO and DUO, corresponding to one and two aliquots of collected plasma samples disks.
2. To ensure enough blood is deposited on the TTPSC, a blade-based lancet is preferred.
3. Detergents such as 1% CHAPS and 1% NP-40 or similar can be added to the buffer to help resolubilize proteins and increase extraction efficacy.
4. Sonication is optional; however, it helps resolubilize proteins and increase extraction efficacy.
5. FAMEs standard mixture may be purchased or prepared in-house following [28] as the more economical choice.
6. If DUO cards are the preferred collection method, be aware that it can be difficult to use collect children's samples and for self-collection in a clinical setting due to the larger amount of volume required. If this issue arises, consider asking study participants to collect two individual UNO cards as it may be easier.
7. Do not band package before use.
8. Rub hands together or under warm/hot water for 3 minutes to warm them up. This will get your blood flowing. You can also move your arms around - fingers should appear pink.
9. Collect blood from your dominant hand as it may be easier to get a large enough drop; it is better to prick the sides of your finger rather than the center.
10. If using the DUO card: apply two large hanging drops, side by side to the rectangular sample port. Ensure that the entire sample port changes from white to red within 30 seconds indicating adequate blood application).
11. While samples may be stored and shipped at ambient temperatures, discs can also be stored at -80°C provided the appropriate equipment is available.

12. Alternatively, you can place the card into a small paper envelope or Ziplock bag; if high humidity is an issue, add a small desiccant bag.
13. Eppendorf tube can be used too – in that case, cut collection disk into small pieces and place them into the tube.
14. This step is optional and helps to recover more sample. If high protein concentration is required, transfer 50 µL originally added to the disk or use acetone precipitation.
15. Steps 4 – 11 are adapted from protocol provided by Bio-Rad.
16. SP3 protocol is adapted from [29].
17. Global profiling protocol listed here is designed for enrichment of polar metabolites and lipids in two, immiscible fractions [30]. If there are other small molecules of interest, please modify accordingly.
18. Store all dried samples at -80°C until further analysis.

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Conflicts of Interest

Jiri Adamec is co-founder of Novilytic and Telimmune. Camila Pereira Braga, Alicia Johnson and Pedro de Magalhães Padilha have nothing to disclose.

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Figures

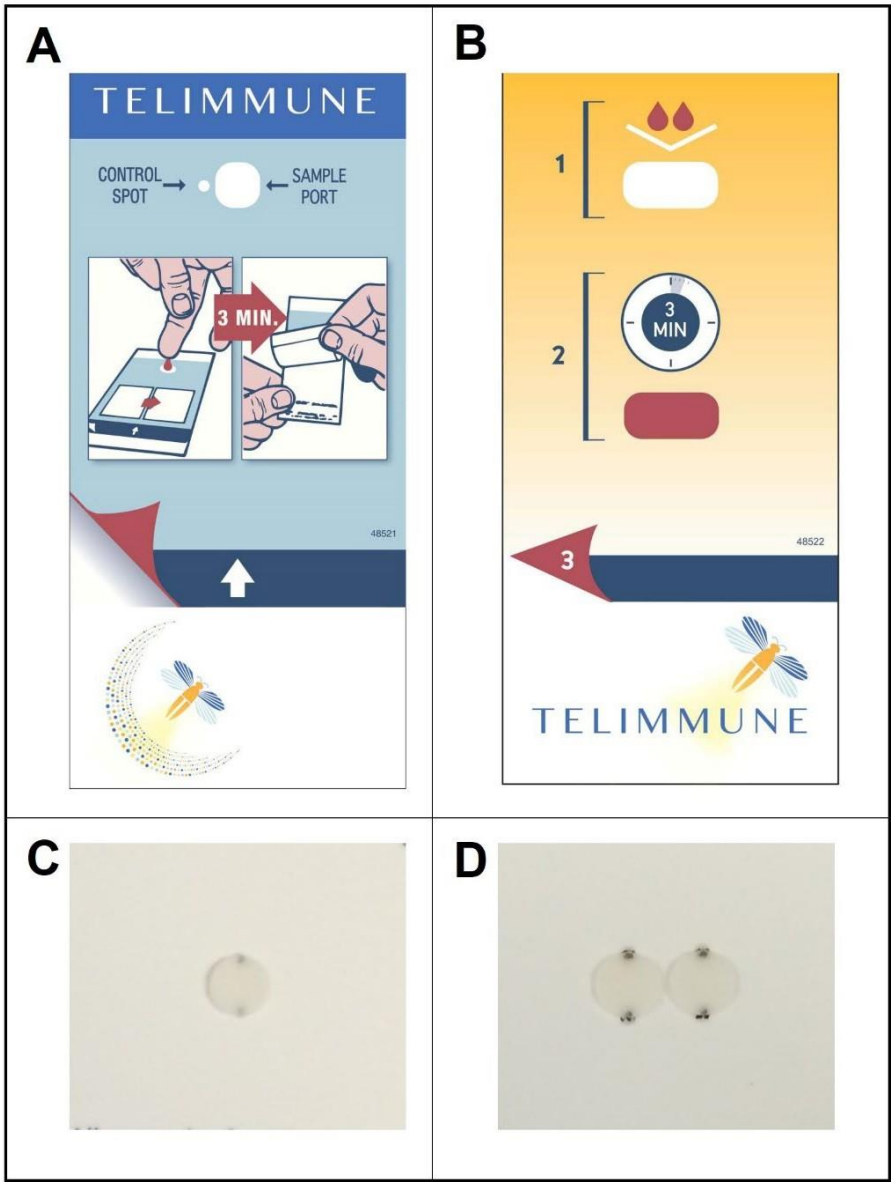

Figure 1. Design and schematic use of Telimmune Plasma Separation Cards: A/ UNO Card with one collection disk (C); B/ DUO Card with two collection disks (D).

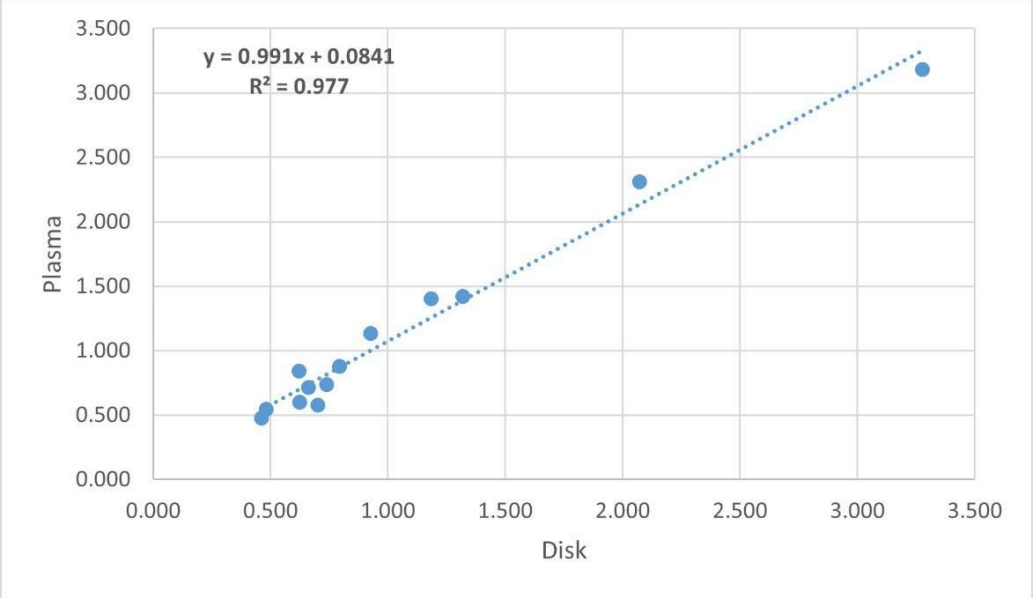

Figure 2. Correlation of ELISA results from SARS-CoV-2 total Ab assay. Disk (dried plasma card) vs venipuncture serology specimens (r=0.98) (adapted from Telimmune).