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Review

Quantitative microsampling for bioanalytical applications related to the SARS-CoV-2 pandemic: Usefulness, benefits and pitfalls

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ABSTRACT

The multiple pathological effects of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and its total novelty, mean that currently a lot of diagnostic and therapeutic tools, established and tentative alike, are needed to treat patients in a timely, effective way. In order to make these tools more reliable, faster and more feasible, biological fluid microsampling techniques could provide many advantages. In this review, the most important microsampling techniques are considered (dried matrix spots, volumetric absorptive microsampling, microfluidics and capillary microsampling, solid phase microextraction) and their respective advantages and disadvantages laid out. Moreover, currently available microsampling applications of interest for SARS-CoV-2 therapy are described, in order to make them as much widely known as possible, hopefully providing useful information to researchers and clinicians alike.

Contents

1. Introduction

The pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), still causing severe illness and death across the world as of June 2020, has been described as the worst

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acute epidemic to hit humanity since the 1918's "Spanish" influenza [1]. The SARS-CoV-2 pandemic is estimated to have caused close to 430,000 deaths and to have infected up to eight million people until now [2], but both tallies are expected to continue to grow for at least some more months, due to the ongoing disease spreading in several parts of the world.

SARS-CoV-2 is an airborne-transmitted virus that infects cells in the respiratory system and causes inflammation and cell death there. The virus then diffuses out in the body and can damage other vital organs, triggering a complex spectrum of pathophysiological changes and symptoms, including respiratory and renal failure, cardiovascular complications, coagulation abnormalities [3].

Due to the large spectrum of possible complications caused by SARS-CoV-2 infections, and to their possible severity, several healthcare, diagnostic and therapeutic activities need to be implemented for optimal disease treatment according to current best practices, including multiple and frequent sampling of biological fluids and tissues. In this situation, logistics and timing of classical laboratory workflows are often strained to the limit, and sometimes cannot accommodate the needs of every patient.

In the last few years, microsampling is emerging in many fields as an attractive alternative to traditional sampling methods entailing the use of relatively large sample volumes, due to its feasibility, increased safety, minimal invasiveness and other advantages [4,5]. Microsampling is usually considered to include any sampling technique that produces specimens suitable for analysis from 100 μL of fluid or less.

1.1. Dried blood spotting (DBS)

Dried blood spotting (DBS) was probably the first dried microsampling technique to be massively used starting in the 1960s, namely for the neonatal screening of congenital diseases (such as phenylketonuria, an inborn metabolism error) [6]. In DBS, a finger (or a newborn's heel) is pricked with a disposable sterile needle and the resulting blood drops are spotted one or more times onto a cellulosic support (roughly similar to filter paper, Fig. 1). The spot is dried and can then be handled with minimal precautions and storage requirements [7–9]. In the following years, DBS was extended, albeit much less frequently, to other biological matrices different from blood; right now, the technique can most accurately be called "dried matrix spotting" (DMS) to reflect its wider usage and applicability. Collectively, DMS represent the large majority of microsampling procedures applied to bioanalysis, with DBS being the most frequent, well-known and widely understood kind [10]. One of the most important issues arising from the application of DMS is sampling volume variability. In fact, when spotting a biological fluid on an absorbing cellulosic support, both matrix viscosity and surface tension influence sample volume and spot area, and these characteristics in turn can produce unwanted and hardly controllable variability in analysis results. Moreover, spotting on paper also produces sample inhomogeneity due to chromatographic effects during fluid absorption [11]. These problems are always present in DBS, due to the nature of the spotting support, the presence of erythrocytes and haematocrit (HCT) variability [12]; however, other DMS kinds can be affected by them. Due to volume variability, DMS usually need special precautions or procedures to produce reliable quantitative results. Over the years, several other microsampling techniques have been developed, proposed and implemented; some of them were direct answers to DMS volume variability and sample inhomogeneity problems, while others were completely unrelated. Among the former, one can cite volumetric absorptive microsampling (VAMS), capillary sampling (such as hemaPEN) and microfluidic spotting (including HemaXis devices). Among the latter, solid phase microextraction (SPME) is available.

1.2. Volumetric absorptive microsampling (VAMS)

VAMS makes use of a device including a plastic handle and a round, calibrated tip, made from a proprietary hydrophilic polymer [13]. The tip is put into contact with the desired biofluid to absorb a constant sample volume into its pores: 10 μL, 20 μL or 30 μL, according to tip size. After drying, the sample absorbed on the tip is ready for storage or pretreatment and analysis (Fig. 2). VAMS allow the microsampling of fixed fluid volumes of whole blood with high reproducibility, and without any significant HCT volume effect [14,15]. The sampling procedure is quite straightforward and can be carried out at home by patients or other people with minimal training. Like most microsampling techniques, VAMS has been developed and validated for blood sampling, but can be applied with satisfactory results to other matrices [16–19].

1.3. Capillary sampling

Suitably calibrated glass capillaries, cut to appropriate lengths, can draw fixed biofluid volumes from a fingerprick [20] or other body locations, irrespective of HCT. Then, the fluid can be spotted onto custom supports for drying or be stored as such until analysis.

A recently developed capillary device (hemaPEN) contains four end-to-end EDTA-coated capillaries that simultaneously collect four identical matrix spot replicates (2.74 μL each) from a single sample. The pen-like design of the device makes it easy to handle, includes a desiccant, grants sample integrity and prevents most contaminations (Fig. 3). It is a single use, tamper-resistant device, needing a specific opening tool for the retrieval of the DMS [21].

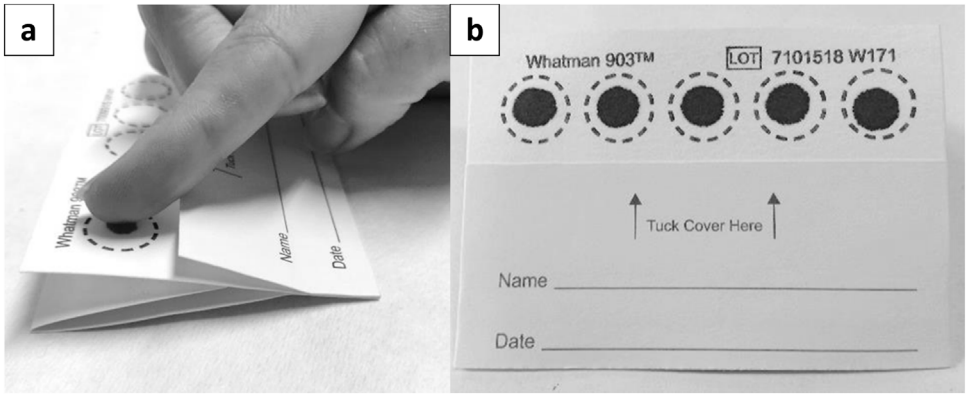

Fig. 1. (a) DBS sampling from a fingerprick and (b) the generated sample replicates deposited on a cellulosic card.

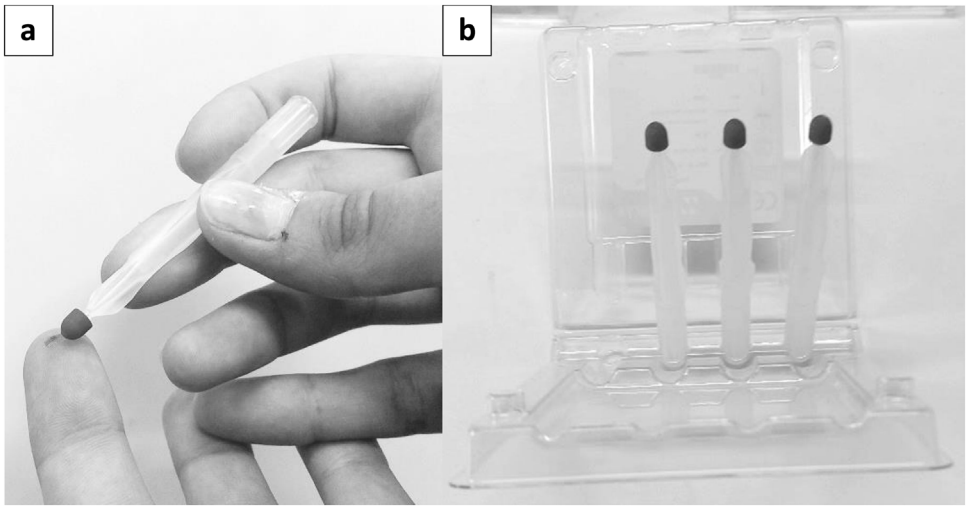

Fig. 2. (a) VAMS sampling from a fingerprick and (b) sampled VAMS placed in the specialised clamshell enclosure for sample drying and subsequent storage.

Fig. 3. (a) Capillary DBS sampling from a fingerprick, (b) in-device filled capillaries before generating DBS and (c) the obtained fixed-volume, pre-cut DBS within the device.

1.4. Microfluidic-based platforms

Different kinds of supports, combinations thereof, and microfluidic designs have been devised to produce dried spots with constant volume, lower inhomogeneity, or derivative matrices.

For example, the Noviplex spotting cards use a two-layer design to obtain one 2.5-μL plasma spot, or two 3.8-μL plasma spots, from a single blood drop. After fingerpricking, the blood drop is deposited on the upper layer, which filters out erythrocytes. The resulting plasma produces calibrated spots on the lower layer [22,23].

HemaXis DB devices can produce fixed-volume 10-μL blood spots on common spotting cards through the use of microchannels engraved in a plastic slab device [24] (Fig. 4). HemaXis DX devices are currently under development, that could directly produce dried plasma spots (DPS) or dried serum spots (DSS) through passive erythrocyte sedimentation in a proprietary microchannel arrangement [25].

1.5. Solid phase microextraction (SPME)

SPME is often considered a miniaturised sample pretreatment technique, rather than a microsampling one. SPME is based on a porous, filamentous fibre (or a solid fibre coated with a porous substance), which is put into contact with, and absorbs, the sample and its components according to their affinity toward the fibre (or coating) material [26]. The analytes can then be selectively desorbed, directly in a gas chromatographic (GC) apparatus, or through solvent extraction in a liquid chromatographic (LC) apparatus. However, the SPME fibre can also be applied directly, *in situ*, to a blood drop from a fingerprick, or to oral fluid, or to other biofluids: in this case, it can be considered a microsampling technique. SPME is thus uniquely positioned, in that it can carry out the simultaneous microsampling and pretreatment of biological fluids. On the other hand, the microfibre operation and handling is not simple, nor devoid of risks, and can be carried out only by specifically trained personnel: this automatically excludes any chance of self- and at-home- microsampling by SPME. Moreover, SPME is a kinetic equilibrium technique, so the sample volume (and the analyte amount) absorbed on the fibre is not easily assessed.

1.6. Advantages of microsampling

Sample volumes in the microlitre range are particularly attractive for invasive sampling techniques (such as blood or cerebrospinal fluid drawing), where the minute volume amounts allow to keep invasiveness to a minimum and to obtain multiple samples within relatively short time spans with a minimum of discomfort, damage and risks for the subject.

Most forms of microsampling also involve sample drying before analysis, and this further extends the benefits of the technique. In fact, fluid sample drying effectively stops, or at least greatly slows down, most chemical and enzymatic degradation processes, thus

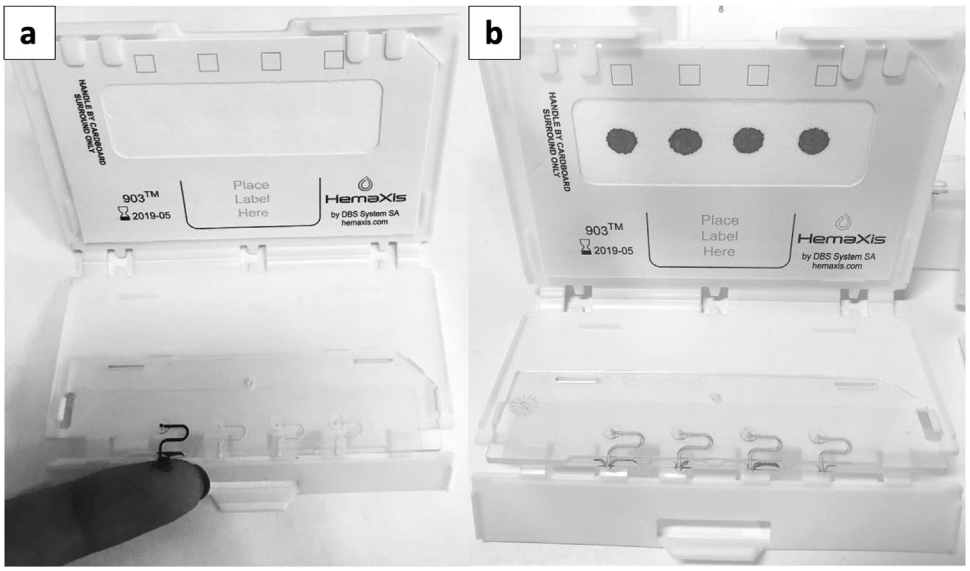

Fig. 4. (a) Fixed-volume DBS sampling from a fingerprick by microfluidic device and (b) the obtained sample replicates on cellulosic support.

often providing extended analyte stability in comparison to fluid samples. This in turn allows extensive sample storage with low space requirements (due to the small sample size) and without stringent temperature control requirements (due to the enhanced analyte stability). These characteristics make dried microsampling increasingly suitable and attractive also for the collection and storage of non-invasive biological fluids, such as urine, sweat, oral fluid and, particularly suited to SARS-CoV-2 infections, epithelial lining fluid (ELF).

Long-term sample storage is not among the most important requirements for biological specimens related to SARS-CoV-2, which on the contrary benefit form very fast turnover and high throughput, due to the need to obtain assay results as soon as possible in time-constrained conditions. However, it can be really useful for applications that could (and will) emerge after the pandemic in the strictest sense has ended, and in particular for research-related needs. For example, retrospective studies could be carried out on microsamples collected during the acute phase of the disease; long-term disease effects or sequelae and their early markers could be studied in this way. As-yet unforeseeable uses for stored biosamples will no doubt be found in the future, and the use of dried microsamples could provide the needed information while at the same time freeing much-needed financial resources and valuable storage space for other applications.

Moreover, as detailed above, most microsampling procedures involve matrix interaction with some kind of support; for this reason, the simple act of microsampling can also be considered a (miniaturised) pretreatment of sort. The resulting microsample is usually easier to purify to the desired degree and needs less complicated, less time-consuming procedures before it is ready for analysis. SPME in particular can be adapted to be a two-in-one microsampling and pretreatment procedure.

The presence of a suitable support also generally aids the automation of analytical workflows [27]. DMS and most other card-based techniques can exploit existing machinery that effect the direct coupling of spotted cards to mass spectrometry (MS) detection, using direct paper spray – MS (PS-MS) interfaces [28]. Alternatively, automated flow-through extraction apparatuses can be used for coupling to chromatographic systems. The VAMS device handle has a similar shape and the same size as automatic pipette tips, so VAMS device racks can be directly inserted in, and handled by, common automatic liquid handling apparatuses [29,30]. SPME fibres can be directly inserted into the mobile phase flow of both GC and LC systems.

As a final, but not less important consideration, microsampling is uniquely positioned to become the technique of choice for at-home- and self-sampling protocols. Their simplicity, safety and independence from specially trained personnel makes most microsampling devices and procedures suitable to obtain reliable results even when carried out in less than ideal conditions. Moreover, the mild storage conditions requirements allow one to safely send microsamples to their destination with minimal precautions and using general, non-dedicated transportation means.

1.7. Disadvantages of microsampling

As already explained, HCT effect is one of the most important and well-known drawbacks of DBS and similar whole blood-based microsampling techniques. Although it has been overcome to some extent with the introduction of alternative techniques (VAMS, microfluidic cards), the large dominance of DBS in the microsampling space means that this is still one of the most pressing problems. Even when the HCT effect proper is not an issue, all whole blood-based microsamples still have to deal with the fact that the sample includes erythrocytes, so that both HCT variability and analyte partitioning between erythrocytes and plasma can affect analysis repeatability. Microfluidic systems that automatically, passively separate plasma from whole blood overcome this problem, but generally at the cost of higher expenses and more complicated setups.

Another pressing problem of microsampling is its lower sensitivity in comparison to classical sampling procedures. Several innovations in the miniaturised pretreatment and analysis space are progressively attenuating this problem, with ever more sensitive instrumentations and ever smaller sample amounts being introduced. For many applications (e.g., monitoring of major biofluid components), the decreased sensitivity can be scarcely relevant, but most cutting-edge science requires equally high sensitivity performances [31]. This drawback is not likely to go away soon, or ever.

The low matrix amounts also mean that relative precision tends to be lower than for higher sample amounts. This disadvantage too is unlikely to disappear soon. Sample handling automation can go a long way to alleviate it, reducing in part operator errors, but

also introducing the need for suitable equipment qualification and validation.

2. Microsampling and SARS-CoV-2

The SARS-CoV-2 pandemic has triggered a healthcare, economic and social crisis that will probably affect most of the world for years to come. Due to the disease novelty, it is currently impossible to predict the end of the pandemic, as well as the possible sequelae and long-term health effects on both symptomatic and asymptomatic patients after recovery.

Due to the nature of the disease, a huge number of people still need, and will need in the future, diagnostic, therapeutic and follow-up tools, measures and facilities that involve some kind of bioanalytical workflow. Microsampling could facilitate the application of these workflows, hopefully making them more practical and feasible, faster and less expensive, while keeping the results equally reliable and useful. An overview of the methodologies potentially useful for SARS-CoV-2 therapy and diagnosis is reported in Table 1.

2.1. Microsampling for diagnostic tools related to SARS-CoV-2

2.1.1. Infection diagnosis

Currently, most SARS-CoV-2 infection diagnoses are carried out either by nasopharyngeal and oropharyngeal swabs, which aim to detect viral RNA, or serologic tests for the analysis of specific antibodies [32,33].

Swab tests are mostly used when the possible infection is presumably in the initial phase and relies on molecular biology techniques, which are carried out on a small amount of nasal or nasopharyngeal fluid, or of expectorate. In this case, a microsampling of sort is already implemented, however its application is currently semi-quantitative at best. In fact, the sample volume is relatively variable according to swab type, due to the different volume and absorbing power of different swab materials; moreover, even when using a single swab type, the absorbing material itself is usually not calibrated for the purpose of volume reproducibility. In this case, some form of DMS could be used after sample retrieval with a swab; or a VAMS procedure could be used directly, using e.g., a modified, longer VAMS handle that can reach the sampling location. However, in the case of VAMS, the very fast tip saturation would be a disadvantage, since it would tend to saturate with nasal or oral fluid before reaching the pharynx. Higher-volume tips, different polymers, or different sampling procedures should be envisaged in this case.

Serologic tests are used during and after the SARS-CoV-2 infection proper, also to diagnose clinical recovery. Rapid serologic tests are available, which provide a purely qualitative result based on an immunoenzymatic assay. They rely on the use of a single blood drop after fingerpricking and represent one of the most established classes of tests using microsampling, as a form of DBS sampling. These are typically either self-sampling kits, or point-of-care kits that need qualified personnel intervention for both sampling and analysis or just for the analysis. Quantitative serologic tests, on the contrary, currently require venepuncture and the drawing of some millilitres of blood. In the context of SARS-CoV-2 quantitative serologic tests, microsampling could constitute an interesting development, if the sensitivity limits can be overcome in the subsequent immunological assay. The usual benefits and drawbacks of microsampling as opposed to large-volume sampling are fully applicable in this case. SARS-CoV-2 antibody quantification can also be carried out on urine, faeces and oral fluid. In all these cases, rapid qualitative tests will probably be highly prevalent. However, if the oral fluid antibody test is confirmed as reliable for diagnosing past SARS-CoV-2 infections, a microsampling approach could provide quantitative results that would also allow evaluating immunity or lack thereof, without resorting to invasive procedures. In this case, at-home sampling could be easily implemented, but the test itself would reasonably be laboratory-based, at least for the time being, also in order to stress method sensitivity and compensate for the minute sample volumes.

Crucially, dried microsampling has demonstrated to stabilise nucleic acids (both DNA and RNA) in a very effective way for years and even decades [34–36], with freezing temperatures (at least −20 °C) greatly improving this parameter for DBS samples. Thus, all dried microsamples collected during the pandemic and afterwards could be stored in relatively small spaces and then be used to study both viral RNA and its host's nucleic acids when the need will arise.

Antibodies are also known to be stable in dried matrices (namely VAMS) at RT for months, and at −20 °C for years [37]. Thus, a perspective on the possible storage and future use of antibodies in dried matrices can be adopted, similar to that on nucleic acids.

2.1.2. Other diagnostic assays

2.1.2.1. Cytokine assays. One of the most severe consequences of SARS-CoV-2 infection is the so-called "cytokine storm syndrome", which can result in dyspnoea, acute respiratory distress syndrome (ARDS), fulminant myocarditis, multiorgan dysfunction and disseminated intravascular coagulation (DIC) [38]. Cytokine quantification can be carried out locally in the respiratory tract, or systemically in blood; it can be useful both to diagnose the syndrome and also to assess the efficacy of anti-inflammatory therapies.

Dealing with samples coming from the respiratory tract or with blood samples, cytokine analysis in the context of SARS-CoV-2 infection diagnosis is conceptually amenable to qualitative and/or quantitative analysis hyphenated with miniaturised sampling strategies such as DMS and VAMS.

Regarding the respiratory tract, a specialized bronchoscopic microsample (BMS) probe can be used to obtain small volumes of ELF, which obviates the need for bronchoalveolar lavage. BMS probes consist of an outer polyethylene sheath and an inner fibre rod probe attached to a stainless steel guide wire. After wedging of the sheath, the probe is guided in distal airways through the bronchoscope channel and BMS is performed for 5–7 secs by exposing the inner fibre rod probe and collecting 12–24 μL of ELF without the need for saline lavage. This procedure has two positive effects: reducing patient distress in comparison to lavage, and also producing samples that are 20–80 times more concentrated [39,40] (a notable exception to the loss of sensitivity observed in other microsampling procedures). This technique can also be useful in research settings for specimen collection in laboratory animals [41]. Another possibility for local microsampling is the collection of breath condensate, which occurs simply by letting the patient's breath pass within a refrigerated glass tube; since patients with severe symptoms are already intubated, this possibility is quite attractive [42].

Cytokine assay in blood is associated with the usual possibility of microsampling application; until now, DBS has been the only applied microsampling technique, and mostly in new-borns or small children [43–45].

Interestingly, cytokine levels can also be assayed in sweat with relatively reliable results; in this case, microsampling by dermal patch has been applied [46].

Finally, interferon α_{2a} has been analysed with in-tube immunoaffinity SPME of plasma [47].

2.1.2.2. Coagulation parameter assays. Several haematic parameters related to coagulation can be useful, or even critical, to diagnose and thus prevent severe or fatal thromboembolic events due to the cytokine storm or to other pathological effects related to SARS-

Table 1
Summary of microsampling applications possibly useful for SARS-CoV-2 diagnosis or therapy.

Microsampling technique	Matrix	Analyte(s)	Sample volume (μL)	Analytical technique	Reference
DBS	Blood	RNA	n.s.	PCR/NB	[34]
DBS	Blood	RNA	n.s.	PCR/NB	[35]
DBS	Blood	HIV-1 DNA	50	PCR/SB	[36]
VAMS	Blood	Antibodies	10	Immunoassay	[37]
BMS	ELF	Cytokines	≅ 20	Immunoassay	[39]
BMS	ELF	Cytokines	2–20	Immunoassay	[40]
BMS	ELF	Cytokines	≅ 10	Immunoassay	[41]
EBC	Breath	Cytokines	≅ 100	Immunoassay	[42]
DBS	Blood	Cytokines	n.s.	Immunoassay	[43]
DBS	Blood	Interferon γ	n.s.	Flow cytometry	[44]
DBS	Blood	Cytokines	n.s.	Immunoassay	[45]
Dermal patch	Sweat	Cytokines	> 400	Immunoassay	[46]
SPME	Plasma	Interferon α_{2a}	n.s.	LC-FL	[47]
BD	Blood	(Prothrombin time)	n.s.	Dedicated device	[48]
BMS	ELF	Peramivir	2–20	LC-MS/MS	[60]
DBS	Blood	Ganciclovir	n.s.	LC-MS/MS	[61]
Noviplex (DPS)	Plasma	Amantadine	40	LC-MS/MS	[62]
DBS	Blood	Camphecene	n.s.	LC-MS/MS	[63]
DBS	Blood	Antivirals	varies	varies	[64]
DBS	Blood	Nevirapine Efavirenz Lopinavir Ritonavir	≅ 100	LC-MS/MS	[67]
DBS	Blood	Ribavirin	10–60	LC-MS/MS	[68]
DPS	Plasma	Nevirapine	100	LC-MS	[69]
DPS	Plasma	Antiviral drugs	50	LC-MS/MS	[70]
DPS (on glass filter)	Plasma	Anti-HIV drugs	100	LC-MS	[71]
FPSE (DOFS)	Oral fluid	NSAIDs	1000	LC-PDA	[74]
BFS (DBS)	Blood	NSAIDs	50–300	LC-PDA	[75]
DBS	Blood	NSAIDs	50	LC-PDA	[75]
SPME	Urine Serum Plasma	Nalidixic acid Diclofenac Naproxen Ibuprofen	n.s.	LC-UV	[76]
SPME	Plasma	Ketoprofen Fenbufen Ibuprofen	n.s.	LC-MS/MS	[77]
SPME	Urine	Naproxen	n.s.	LC-UV	[78]

(continued)

Microsampling technique	Matrix	Analyte(s)	Sample volume (μL)	Analytical technique	Reference
DBS	Blood	Endogenous glucocorticoids	60	LC-MS/MS	[79]
VAMS	Blood	Endogenous glucocorticoids	10	LC-MS/MS	[79]
Noviplex (DPS)	Plasma	Endogenous glucocorticoids	40	LC-MS/MS	[79]
DBS	Blood	Endogenous glucocorticoids	n.s.	LC-MS/MS	[80]
DBS	Blood	Endogenous glucocorticoids	n.s.	LC-MS/MS	[81]
DBS	Blood	Endogenous glucocorticoids	n.s.	LC-MS/MS	[82]
SPME	Urine	Endogenous glucocorticoids	n.s	LC-MS/MS	[83]
SPME	Urine	Endogenous glucocorticoids	n.s.	LC-UV	[84]
DBS	Blood	Budesonide	n.s.	UHPLC-MS/MS	[85]
SPME	Tissue	Methylprednisolone	n.s.	LC-MS/MS	[86]
SPME	Plasma	Exogenous glucocorticoids	n.s.	LC-MS/MS	[87]
DUS	Urine	Dexamethasone Glucocorticoids	n.s.	LC-MS/MS	[88]
VAMS	Urine	Dexamethasone Glucocorticoids	30	LC-MS/MS	[88]
DBS	Blood	Tacrolimus Cyclosporin A	n.s.	LC-MS/MS	[89]
DBS	Blood	Tacrolimus	10	PS-MS/MS	[90]
DBS	Blood	Immunosuppressants	n.s.	LC-MS/MS	[91]
DBS	Blood	Tacrolimus	n.s.	LC-MS/MS	[92]
DBS	Blood	Tacrolimus	n.s.	LC-MS/MS	[93]
DBS	Blood	Immunosuppressants	n.s.	SPE-MS/MS	[94]
HemaXis (DBS)	Blood	Tacrolimus Mycophenolic acid	10	LC-MS/MS	[95]
VAMS	Blood	Monoclonal antibodies	20	Immunoassay	[96]
DBS	Blood	Monoclonal antibodies	n.s.	Immunoassay	[96]
VAMS	Blood	Tacrolimus	10	LC-MS/MS	[97]

(continued)

Microsampling technique	Matrix	Analyte(s)	Sample volume (μL)	Analytical technique	Reference
VAMS	Blood	Immunosuppressants	10	LC-MS/MS	[98]
VAMS	Blood	Tacrolimus	10	UHPLC-MS/MS	[99]
VAMS	Blood	Tacrolimus	n.s.	LC-MS/MS	[100]

Table 1 (*Continued*)

(continued)

Microsampling technique	Matrix	Analyte(s)	Sample volume (μL)	Analytical technique	Reference
DBS	Blood	Tacrolimus	n.s.	LC-MS/MS	[100]
DMKS	Milk	Tocilizumab	≅ 30	Immunoassay	[101]
DBS	Blood	Heparin	n.s.	LC-MS/MS	[103]
DBS	Blood	Chloroquine	75	LC-UV	[106]
DBS	Blood	Chloroquine	n.s.	LC-UV	[107]
DBS	Blood	Chloroquine	n.s.	LC-UV	[108]
DBS	Blood	Chloroquine	80	LC-UV	[109]
DBS	Blood	Chloroquine	15	LC-MS/MS	[112]
DBS	Blood	Antimalarials	n.s.	LC-MS/MS	[113]
DBS	Blood	Chloroquine	n.s.	LC-HRMS	[114]
VAMS	Blood	Hydroxychloroquine	10	LC-MS/MS	[115]
DBS	Blood	Hydroxychloroquine	n.s.	LC-MS/MS	[116]
DBS	Blood	Li^+	50	GF-AAS	[118]
DPS	Plasma	Li^+	20	GF-AAS	[118]
DBS	Blood	Cyclosporin A	n.s.	LC-MS/MS	[120]

Abbreviations: AAS, Atomic Absorption Spectrometry; BD, Blood Drop (not dried); BFS, BioFluid Sampler; BMS, Bronchoscopic MicroSample; DBS, Dried Blood Spots; DMKS, Dried MilK Spots; DPS, Dried Plasma Spots; DOFS, Dried Oral Fluid Spots; DUS, Dried Urine Spots; EBC, Exhaled Breath Condensate; ELF, Epithelial Lining Fluid; FL, FLuorescence detection; FPSE, Fabric-Phase Sorptive Extraction; GF, Graphite Furnace; HRMS, High-Resolution Mass Spectrometry; LC, Liquid Chromatography or high-performance Liquid Chromatography; MS/MS, tandem Mass Spectrometry; NB, Northern Blotting; n.s., not specified (for BD and DBS it usually ranges from 50 to 200 μL); NSAID, Non-Steroidal Anti-Inflammatory Drug; PCR, Polymerase Chain Reaction; PS, Paper Spray; SB, Southern Blotting; SPE, Solid-Phase Extraction; SPME, Solid-Phase MicroExtraction; UHPLC, Ultra-High-Performance Liquid Chromatography; VAMS, Volumetric Absorptive MicroSampling.

CoV-2 infection. Several examples of microsampling for this kind of purpose are available. For example, point-of-care (POC) instruments have been developed to assess prothrombin time, which work by the direct analysis of the coagulation of a single blood drop [48].

2.2. Microsampling for therapeutic tools related to SARS-CoV-2

Due to the large array of different pathological effects the SARS-CoV-2 can cause, several kinds of pharmacological therapies can be applied, and the choice of the specific drug and dose would be better tailored to each individual patient. In this regards, therapeutic drug monitoring (TDM) is one of the most effective practice that allow treatment personalisation and optimisation based on objective measurements [49–52].

TDM includes the repeated determination of drugs and metabolites plasma levels, together with the use of chemical-clinical correlations (i.e., correlations between administered drug dose and plasma levels; between plasma levels and therapeutic efficacy; between plasma levels and side and toxic effects) [53–55].

The information thus obtained represents a sound, rational and objective foundation, on which the clinician can base its activity supplementing clinical observations to build a safe and effective therapeutic platform [56]. TDM can also lead to reduced healthcare expenses, due to the possibility of better efficacy, increased patient compliance and enhanced safety, leading to a reduction in hospitalizations due to unwanted effects or therapy ineffectiveness [57]. TDM is particularly useful in avoiding overdoses and their consequences, as well as in managing drug-drug interactions (DDI) [58]: of course, this is even more important during polypharmacy (i.e. concurrent multiple-medication regimens).

2.2.1. Antiviral therapy

An effective antiviral therapy could solve most problems related to SARS-CoV-2 infections. Unfortunately, until now most antivirals seem to have produced improvements in patients' clinical conditions only when administered as part of complex therapeutic regimens including multiple drugs [59].

As one could expect, BMS for ELF microsampling has been used for the analysis of antivirals (peramivir) administered systemically [60]. However, generally speaking, apart from this single example DBS DPS have been the only microsampling variants to be applied [61–64].

Specifically, the most frequent antiviral therapies applied for SARS-CoV-2 infections involve lopinavir (often associated with ritonavir), remdesivir, favipiravir, ribavirin and arbidol [65]. To date, papers have been published on the determination and monitoring of lopinavir, ritonavir and ribavirin [66–68] in DBS and also in DPS [69,70]. A peculiar example of DPS on glass filters instead of paper ones has been reported [71]; it is unclear which advantages and disadvantages this substrate brings to the assay.

On the contrary, no microsampling technique seems to have been applied to remdesivir, favipiravir and arbidol determination.

2.2.2. Anti-inflammatory and immunosuppressive therapy

In the framework of SARS-CoV-2 treatments, this kind of therapy aims to avoid or reduce the impact of the cytokine storm, thus preventing the damage caused by the excessive immune response, using anti-inflammatory and immunosuppressant agents. Among the former, one can cite the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen and ketoprofen and the corticosteroids methylprednisolone, dexamethasone and budesonide; among the latter, one can cite tocilizumab and tacrolimus [72,73].

2.2.2.1. NSAIDs. Since NSAIDs are among the most widespread drugs in the whole world, several applications involving their determination in biological microsamples have been published.

A variant of DMS has been developed, which involves the preparation of dried oral fluid spots (DOFS) using fabric phase sorptive extraction (FPSE) [74]. This technique uses a calibrated-size patch of synthetic fabric, instead of cellulose-based paper, to absorb reproducible amounts of oral fluid. A similar workflow has also been applied to blood [75], but using calibrated biofluid samplers (BFS) made from a cellulose substrate coated with a porous sol-gel sorbent; due to the sampler architecture, sample volume can be varied up to 1 mL, obtaining considerable advantages in term of sensitivity (balanced of course by a corresponding loss in practicality, minimal invasiveness and storage space requirements).

A few SPME applications to NSAIDs have also been published over the years [76–78], however most of them are proofs-of-concept for new kinds of absorbing materials [76,77] and just a few were applied to real samples from patients [78]. Moreover, the latter deals with a SPME application as a sample pretreatment procedure on conventional fluid samples, not a combination of

microsampling and miniaturised pretreatment, thus being limited as an ïn-labšample preparation technique.

2.2.2.2. Corticosteroids. The performances of three different microsampling techniques (DBS, VAMS, DPS through Noviplex cards) have been compared for the analysis of endogenous corticosteroids, although the process has been applied to rat blood, not to human samples [79]. DPS had the advantage of producing results directly comparable to those of liquid plasma, while VAMS and DBS results, and DBS sampling volume, are influenced by haematocrit. On the other hand, the larger sampling volume of VAMS (10 μL vs. 3.8 for DPS and 7.5 μL for DBS) confers higher sensitivity to this assay.

In general, microsampling applications have been reported for endogenous corticosteroid determination (thus including cortisol and/or cortisone) in DBS [80–82] and in urine pretreated by SPME [83,84]. Regarding specifically the exogenous corticosteroids most often involved in SARS-CoV-2 therapy, budesonide has been analysed in DBS [85]. SPME procedures are also available: methylprednisolone has been directly microsampled by SPME in situ during liver surgery [86], but similar procedures could be envisioned for respiratory tract sampling; automated thin-film SPME on plasma has been applied to the analysis of dexamethasone, budesonide and prednisolone [87].

Dexamethasone is currently being evaluated as one of the most promising agents for SARS-CoV-2 infection treatment. A microsampling procedure for its determination in dried urine spots (DUS) and urine VAMS is available [88], which also includes other exogenous and endogenous glucocorticoids.

2.2.2.3. Immunosuppressants. Many immunosuppressants have a rather narrow therapeutic window and are mostly used chronically for many years. As a consequence, their monitoring is quite widespread, and several microsampling procedures are available. As usual, DBS is the most frequent microsampling approach [89–93]. In one study, according to the authors, heated flow-through desorption allowed to obtain HCT-independent recovery of the analytes [94]. In another study, fixed-volume DBS were obtained by means of an HemaXis device [95].

A comparison of blood VAMS and DBS for the monitoring of tocilizumab and six other therapeutic monoclonal antibodies showed that both techniques provided high analyte stability for at least 1 month at room temperature [96]. Blood VAMS has also been applied for tacrolimus quantification [97–99], including a comparison study between VAMS and DBS [100], which has found better agreement with whole blood tacrolimus levels for the latter than for the former.

A peculiar microsampling application is the use of dried milk spots (DMKS) for the monitoring of tocilizumab in breastfeeding mothers [101], which introduces the possibility of also monitoring infant exposure during the mother's treatment.

2.2.3. Anticoagulant therapy

Injective anticoagulants (mainly low molecular weight heparin, LMWH; or unfractionated heparin, UFH) are suggested as possible therapeutic interventions in SARS-CoV-2 patients who are at risk for thromboembolic events (see above), or who already were before the infection [102]. Microsampling approaches to heparin analysis are few and far between: DBS from new-borns of heparin-treated mothers have been used to search for possible heparin presence, but no positive results has been obtained [103]. Oral anticoagulants are considered too prone to drug-drug interactions and too subject to monitoring needs to be useful in a clinical setting of SARS-CoV-2 treatment; of course, the latter could be a good reason to study and propose new, straightforward and practical microsampling approaches that would make their TDM more feasible.

2.2.4. Other investigational therapies

2.2.4.1. Chloroquine and hydroxychloroquine. Although mainly used against malaria, chloroquine and hydroxychloroquine have demonstrated efficacy against several viruses, including coronaviruses [104]. They are also used as anti-inflammatory/immunosuppressant agents in autoimmune diseases [105], so they could provide multiple beneficial biological activities to SARS-CoV-2 patients.

DBS application to chloroquine analysis started in 1985, with the first paper published on this topic by Lindstrom et al. [106]; several other applications have been reported over the years [107–109] (as also reviewed in Taneja et al., 2013 [110] and in Casas et al., 2014 [111]), and until recently [112–114].

Regarding hydroxychloroquine, VAMS has been used for its determination in rheumatoid arthritis patients [115], while DBS has been used for a pharmacokinetic study in rats [116].

2.2.4.2. Lithium salts. Lithium salts, usually administered to treat bipolar disorder, have also demonstrated some antiviral activity in preclinical studies [117]. Lithium requires constant TDM during the therapy, so it is a prime candidate for microsampling application; however, until now just DBS and DPS have been used for this purpose [118].

2.2.4.3. Cyclosporin A. Cyclosporin A is well known immunosuppressant agent that also has activity against coronaviruses [119], so it could benefit SARS-CoV-2 patients with two different mechanisms. Until now, DBS [120] and VAMS [98] have been the only two microsampling technique applied to cyclosporin A.

2.2.4.4. Camostat. Camostat is a recent protease inhibitor agent that could be useful to prevent SARS-CoV-2 virus' entry into the cell, effectively preventing the infection. Until now, no microsampling procedure for camostat analysis has ever been published.

3. Conclusion

The SARS-CoV-2 pandemic has taken the whole world aback, and scientists and clinicians alike are currently struggling to find new diagnostic and therapeutic tools that could help patients to successfully cope with this multi-faceted and polymorphous disease. Within this landscape, microsampling could prove to be uniquely positioned to provide reliable quantitative information in short times and with high throughput, when coupled to both chemical and biochemical analytical tools. Moreover, dried microsampling could prove an invaluable asset in cheaply and practically preserving biological specimens for future use.

Until now, simple DBS on common cards has been by far the microsampling technique of election for most bioanalytical applications; however, its drawbacks have spurred the birth of a wealth of modified or alternative procedures that are now reaching maturity, including DMS, VAMS and microfluidic and capillary matrix spotting. Taken together, all these microsampling techniques could prove to be even more useful, reliable and customizable than DBS itself. The corresponding applications potentially useful for SARS-CoV-2 therapy are surmised in Table 1.

The future hopefully holds aetiological, resolutive SARS-CoV-2 therapies that could be personalised to each patient's peculiar needs and individualised responses. Microsampling could be a decisive factor in accelerating the coming of this future and in making it widely applicable, with reduced costs and increased effectiveness.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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