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Model fluids representing aqueous *in-vitro* diagnostic reagents for the development of dispensing systems

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Analyzers for in-vitro diagnostic (IVD) testing facilitate the determination of medical information from biological samples. To reach a high quality, the detection reagents have to be dispensed with a high degree of precision and accuracy. A technology change from conventional pipetting systems to contact-free dispensers provides the opportunity to reduce carry-over and handle reagents in the microliter range. A great challenge for the development and validation of new systems is the huge variety of the IVD reagents. This work presents the fluidic properties of 646 different aqueous IVD reagents and how they can be represented by a set of easy-to-prepare model fluids, covering the rheological range of the reagents. In addition, based on the model fluids, a standardized approach is presented for the evaluation of dispensers for IVD applications.

Introduction

In-vitro diagnostic (IVD) testing has advanced over the past decades starting with manual tests, followed by robotic pipetting and then automated batch processing up to high-throughput screening applications today. Permanent challenges for the improvement of fluid handling in IVD systems are the reduction of carry-over, decreasing waste, improved precision and reduced reagent consumption [1]. However, there are still needs for improvements today. New guidelines for the precision of IVD tests, the aim of higher performance, lower costs and reduced turnaround times are reasons for running further investigations. In all development steps new technical solutions can be beneficial. For example, contact-free dispensing systems could be the next step in the chain of IVD testing, because they are a great opportunity to reduce carry-over and reduce the reagent consumption owing to the feasibility of dispensing small volumes with high accuracy and precision [2] (http://www.microtec-suedwest.de/der-cluster/leitthemen-und-projekte/leuchtturm-in vitro-diagnostik/).

IVD applications determine different parameters from biological samples such as blood, plasma, serum, urine, among others. To ensure a detectable amount of analyte within the sample, volumes in the microliter or submicroliter range are required. The volumes of the detection reagents are in the range of the sample volumes, or higher which means that systems for picoliter dispensing such as inkjet technology or acoustic fluid transport are not suitable. Common dispensing technologies fulfilling the demand are, for example, positive displacement systems (PDS) and time–pressure-dependent systems (TPS).

In the area of IVD applications all developments include challenges caused by a broad range of different fluids with a variety of physical properties. For example, one of the brand leaders in the diagnostic sector includes nearly 300 different tests of mostly two or three reagents each in its portfolio. This complexity is a special challenge when new dispensing technologies are established and the possibility to operate all reagents has to be demonstrated. For the introduction of a new dispensing system the requirements have to be clearly defined [3]. Requests on the workflow, the hardware and the resulting performance of dispensing IVD reagents are usually well described, for example

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by IVD guidelines (e.g. DIN EN ISO 15197) or by the customers. Reliable sources for the ranges of the IVD reagent properties are not easily accessible. However, using contact-free dispensers the reagent properties define the energy and the dynamics needed to eject a droplet or jet from the orifice. The conditions for the fluid breakup are described by the Weber number (*We*), which is defined as the ratio of surface tension energy (E_σ) to kinetic energy (E_{kin}) [4] (Eq. (1)).

$$We = \frac{1}{12} \cdot \frac{E_{kin}}{E_\sigma} = \frac{\rho v^2 d}{\sigma} \quad (1)$$

The *We* depends on a characteristic length, which in this case is the diameter (d) of the circular orifice, the fluid density (ρ) and the surface tension (σ), as well as the fluid velocity (v). A droplet breakup occurs at the critical *We* (We_c) if the kinetic energy overcomes the surface tension energy. For the calculation of We_c the Ohnesorge number (*On*) (Eq. (2)) is required, which describes the dependency of the We_c on the fluid viscosity (η) [4].

$$On = \frac{\eta}{\sqrt{\rho \cdot d \cdot \sigma}} \quad (2)$$

An empirical functional correlation between We_c and *On* is defined in Eq. (3) [5]:

$$We_c = 12(1 + 1.77 \cdot On^{1.6}) \quad (3)$$

For dispensing jets or droplets, the breakup conditions are reached at $We_c \geq 8$ or ≥ 12 [6]. However, regardless of dispensing droplets or jets, the main influencing energy terms used to eject the fluid from the orifice are the kinetic energy $E_{kin} \sim \rho v^2$, the viscous dissipation $E_d \sim \eta/d^2$ and the surface tension energy $E_\sigma \sim 4\sigma/d$. They include the density, the viscosity and the surface tension of the fluid. Relating to the IVD reagents, these are the important parameters, which have been used to evaluate the dispensing behavior of a contact-free dispensing system. To ensure that the dispenser can handle all reagents it seems that the whole range has to be tested. This requires a great effort so that a characterization with all IVD reagents results in a disproportionate effort in cost and time.

To ensure a meaningful characterization with fewer fluids, this case study presents seven model fluids (MFs) covering the fluid properties of the 646 investigated IVD reagents. How these fluids can be used for a standardized dispenser evaluation for IVD applications is shown using two different dispensing technologies. The procedure includes five steps, evaluating the suitability of the dispenser for contact-free release of IVD reagents and describing its dependency on the fluid properties. The study refers to all developers of IVD dispensing systems for applications in immunochemistry, clinical chemistry and molecular diagnostics that are looking forward to a fast and straightforward tool for dispenser evaluation.

Determination of the IVD reagent properties

Measurement methods

The densities of the liquids were measured at 20°C using a pycnometer (Brand, Gay-Lussac type, 10 ml) with a measurement error of the system of approximately ±3%. A double-cone plate system from Haake (Rheostress 600, DC60/1° with cover) was used to determine the viscosity at a shear rate of 492 s^{-1} at 20°C (error ≤±5%). Additionally, a shear rate ramp between 200 s^{-1} and 2000 s^{-1} was measured to identify non-Newtonian behavior. For shear rates above this a capillary viscometer from Rheosense (mVROC) was used. The surface tension was measured with the Krüss EasyDrop system using the pendent drop method [7,8]. The typical system error is assumed to be ±5%.

IVD reagent properties

A representative set of IVD reagents was measured to investigate the range of the reagent properties [density (ρ), viscosity (η) and surface tension (σ)]. An overview of the currently available tests for clinical chemistry, immunochemistry and molecular diagnostics supplied by Roche Diagnostics can be found at the Cobas® website (http://www.cobas.com/home/analytes.html). Each test consists of up to three different reagents, so that the overall number of reagents is higher than the available number of tests. Densities of 646 IVD reagents were measured to investigate the range from 997 to 1192 kg/m^3. The distributions of the measured values are shown in Fig. 1a. Most of them are less than 1050 kg/m^3 and only 12 are greater than 1100 kg/m^3.

The viscosity of the investigated IVD reagents varies between 0.87 and 15.6 mPas. As can be seen in Fig. 1b, the majority of the reagents features viscosities less than 2.0 mPas, nearly 50 reagents are between 2.0 and 4.0 mPas and only a few values are above this. Eight fluids feature pseudoplastic behavior with decreasing viscosities at increasing shear rates. For the IVD reagents the viscosities are investigated at shear rates between 200 and ~45 000 s^{-1}. The results are plotted in Fig. 1b. The surface tensions of the investigated reagents vary between 26.2 and 77.8 mN/m. All values are evenly distributed (Fig. 1c), hence there is no range containing a particularly high or low number of measurement points.

Development of MFs representing the IVD reagents

The rheological data of the IVD reagents are the basis for the development of different MFs covering the properties of the IVD reagents. To show their position in the IVD reagent landscape (Fig. 2), a viscosity–surface-tension plot is used. Therein the densities of the fluids are neglected, because of the small variations over the whole measured spectrum. The MFs are positioned almost at the corners (MF I–IV). The highest and the lowest surface tensions are not exactly covered, because they are the result of the interaction of different components (such as proteins, salts and sugars). To keep the MFs as simple as possible an adaption to these values was not conducted. However, to reach the highest measured surface tension the addition of 15% (w/w) NaCl to water is sufficient [9]. Moreover, viscous additives could lower the surface tension, which is the case for MF IV and the presented higher viscous IVD reagents.

Although, there is no measured reagent in the lower left corner of the landscape, MF III is defined to cover a quadratic space. Moreover, it is not unlikely that a new formulated reagent features these properties, because addition of a detergent is sufficient to switch the surface tension. Beside the quadratic space, three more fluids were prepared: MF V representing the IVD reagents with pseudoplastic behavior, MF VI in the highest distribution and MF VII almost in the middle of the landscape. To adjust the desired rheological properties of the MFs, glycerol and different typical nonionic detergents are used. All detergent concentrations are above the critical micelle concentrations to offer reproducible preparation, and to avoid changed values caused by surface contacts. An additional adjustment of the density is not necessary, because the densities of the MFs already cover the range of the IVD reagents (except for the highest one). The components of the MFs and the resulting fluid properties are presented in Table 1.

Comparison: dispensing IVD reagents and MFs

A TPS (Fig. 3) is a system where the dispensed volumes are influenced by the fluid viscosity and density is used to evaluate the comparability between the MFs and the IVD reagents. Several of the IVD reagents with rheological properties very close to a MF (Table 2) are dispensed with the same setup as the corresponding MF. So, the dispensed volumes of the complex IVD reagents are directly comparable to the simple MFs. The

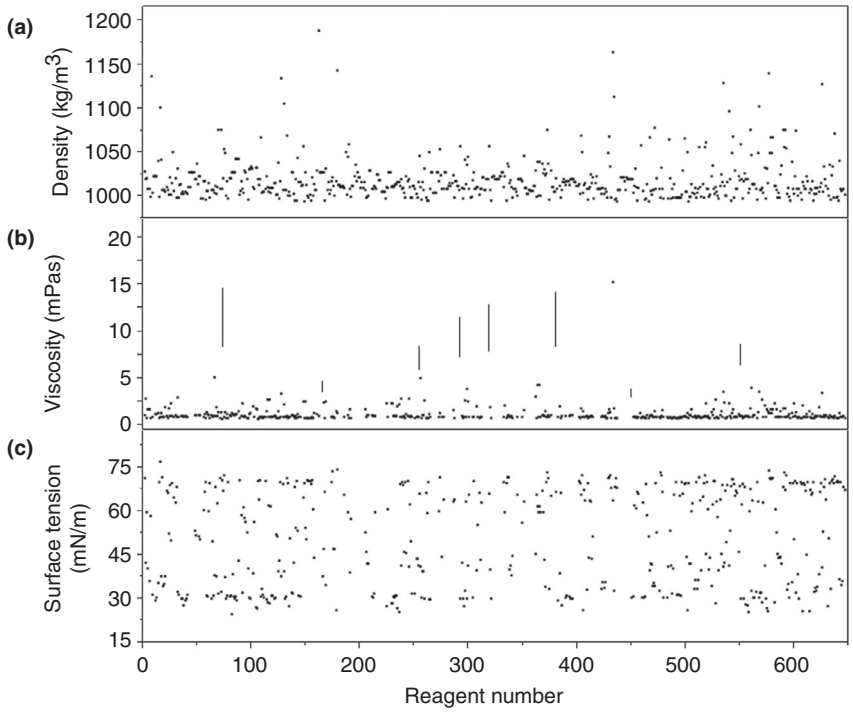

FIGURE 1

Range of **(a)** density, **(b)** viscosity and **(c)** surface tension of a set of representative *in-vitro* diagnostic (IVD) reagents. Lines in (b) represent fluids with shear-rate-dependent viscosities for values measured between 200 s^{-1} and ~45 000 s^{-1}.

results show that the released volumes of the IVD reagents fit to the target volume of the corresponding MF within the system error (2.8%) of the dispenser. For visual representation the dispensed volumes of the MF are set to 100% and compared to volumes of the MFs in Fig. 4. Thus, it is shown that the components of the reagents feature no additional influence on the dispensed volumes. Hence, if the properties of fluids with completely different components are similar, the dispensing results are also similar (within the system error). For further dispenser characterization this is an important fact because it offers the possibility of using simple fluids instead of complex mixtures. To measure the dispensed volumes an ultramicrobalance from Mettler Toledo (XP2U) combined with the software described by Liang *et al.* [10] was used.

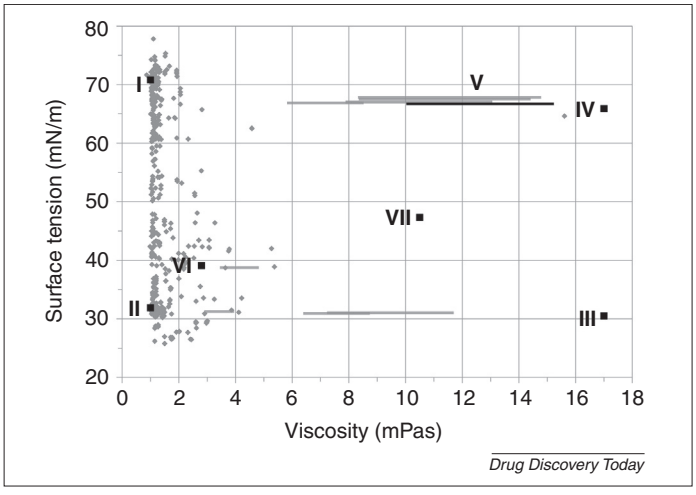

FIGURE 2

Model fluids (MFs) I–VII in the landscape of the *in-vitro* diagnostic (IVD) reagents. Lines represent fluids with shear-rate-dependent viscosities for values measured between 200 s^{-1} and ~45 000 s^{-1}. A more detailed description of the MFs is shown in Table 1.

Standardized approach: dispenser characterization using the MFs

With the help of the MFs a dispenser evaluation was conducted to define the dependency between the dispensed volumes and the fluid properties, as well as to evaluate the ability of the dispenser for contact-free ejection of the whole IVD reagent spectrum. The used dispensing systems (PDS and TPS) are described below.

Dispensing systems

As representative for the PDS, the cartridge for dispensing a fluid was chosen (European patent application EP12167108.5). This system features an accuracy of 0.2% and a coefficient of variation (CV) of 0.3% for dispensing 1 μl water over half a day (eight runs, 21 dispenses each). The working principle of the system could be described in four steps, which are visible in Fig. 5. During the home position the valve opening is directed to the fluid reservoir and the piston stroke is at the zero position (Fig. 5a). For fluid intake, the desired fluid amount is transported into the two-way valve while the piston moves backwards (Fig. 5b). Then, the opening side of the valve is switched to the direction of the nozzle (Fig. 5c) by rotation. In the final step (Fig. 5d) the fluid is ejected while the piston moves into the zero position with a set velocity of $v = 0.25$ m/s and an acceleration of

TABLE 1

Components, amounts and rheological properties of model fluids (MF) representing the properties of the IVD reagents at 20°C

MF	Component	Supplier	Amount (%)	Viscosity (mPas)	Surface tension (mN/m)	Density (kg/m^3)
I	Deionized water			1.0	70.8	998
II	Deionized water			1.0	31.9	998
	Sympatens-AL/090 P (RD)	Kolb AG – Hedingen (CH)	0.2 (w/v)			
III	Deionized water			16.9	30.5	1169
	Sympatens-AL/090 P (RD)	Kolb AG – Hedingen (CH)	0.2 (w/v)			
	Glycerol	VWR (≥99.5%)	66 (w/w)			
IV	Deionized water			16.9	65.9	1169
	Glycerol	VWR (≥99.5%)	66 (w/w)			
V	Deionized water			8.6–13.4	66.7	1003
	Kollidon® 90 F	BASF	3.6 (w/v)			
VI	Deionized water			2.8	39.1	1078
	Tween® 20	Croda GmbH – Nettetal (DE)	0.21 (w/v)			
	Glycerol	VWR (≥99.5%)	32 (w/w)			
VII	Deionized water			10.5	47.3	1139
	Myrj® S100	Croda GmbH – Nettetal (DE)	2 (w/v)			
	Glycerol	VWR (≥99.5%)	55 (w/w)			

$a = 30\ m/s^2$. In the literature [11] several factors influencing the dispensed volume of PDS are described. These are, for example, the dispensing time, the fluid level and the fluid behavior. However, with the cartridge used here for dispensing a fluid no influence on the dispensed volume can be observed. The reason could be that the system operates with only the desired volumes in the syringe, contrary to other systems that always operate with a much greater volume.

The second system used is a TPS. The volume-determining factors are the pressure on the fluid and the open time of the valve. Depending on the parameter settings, the system can dispense small droplets or jets. Fluids with different viscosities and densities result in different friction forces (within the fluids and at the wall), which in turn result in uneven counterforces against the applied pressure. Therefore, the dispensed volumes depend on the fluid properties. An example of a TPS is a commercially available system (Vermes Microdispenisng, MDS 3000 Series, MDV 3010-70) with an accuracy of 0.5% and a CV of 0.4% for dispensing 1 µl water over one day (11 runs, 21 dispenses each). The dispensed fluid volume can be adjusted by the pressure, the dimensions of the nozzle and the lift of the needle. A sketch of the system is shown in Fig. 3, or on the Vermes Microdispensing website (http://www.vermes.com/en/).

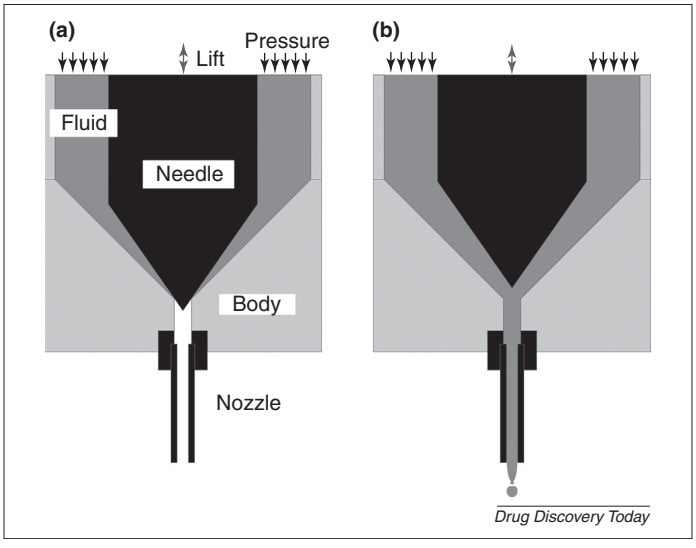

FIGURE 3

Working principle of the Vermes dosing system (Vermes Microdispensing, MDS 3000 series, MDV 3010-70). **(a)** Home position and **(b)** fluid ejection due to needle lift and pressure.

Evaluation in five steps

The standardized dispenser evaluation is presented here for the two types of dispenser for a target volume of 1 µl. Each measurement point is evaluated with three runs of 24 dispenses each. The CVs of all presented measurement results are <0.9% for the PDS, and <3.0% for volumes less than 250 nl and <1.7% for volumes above 250 nl for the TPS.

Step 1: evaluation of the dependency on fluid surface tension by dispensing MF I and II with different surface tensions and identical viscosities

If MF I and II are dispensable with equal results and under the same operating conditions the system works independently of the surface tension. If MF II is dispensable and MF I is not the required energy for contact-free ejection is not reached. This can be explained by the We defining the required energy for contact-free dispensing, which is reciprocally proportional to the surface tension. The PDS and the TPS can dispense the MFs (I and II) with the target volume and a parameter setup 1. The surface tension difference of 38.9 mN/m between the MFs does not affect the dispensed volume (Fig. 6a and b).

Step 2: evaluation of the dependency on fluid viscosity and density by dispensing MF II and III

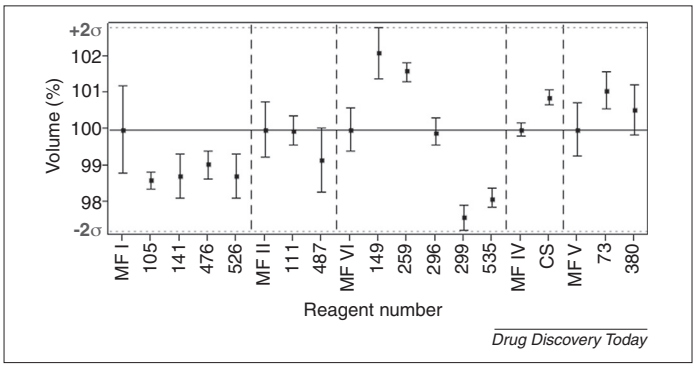

FIGURE 4

Dispensing results of some model fluids (MFs) in comparison with *in-vitro* diagnostic (IVD) reagents of identical viscosity and density, dispensed with the Vermes dosing system within the system error of 2.8% (2σ). The dispensed volume of the appropriate MF is set to 100%. A more detailed description of the reagents is shown in Table 2.

with different viscosity and density and constant surface tension

Viscosity and density depend on each other. The more viscous MFs also feature higher densities. For further investigations both are evaluated together, whereas the range of the viscosity is much larger than the range of the density. If both MFs (II and III) are dispensable with equal results the system does not depend on fluid viscosity and/or density. The PDS system can dispense the MFs II and III with the same parameter setup 1, as would be expected for a PDS (Fig. 6c). However, the TPS needs a modified parameter setup 2 to reach the target volume of 1 μl for MF III (Fig. 6d). So, the TPS shows a dependency of the dispensed volume on the viscosity and/or density of the used fluid and the PDS does not.

Step 3: evaluation for dosing of the whole Newtonian fluid landscape by dispensing MF IV with a combination of a high viscosity, density and surface tension

For evaluation of the whole fluid landscape of Newtonian IVD reagents a further check with fluid IV is recommended. Because the viscosity, density and the surface tension of MF IV are high, the energy required to release a droplet or a jet is the highest of all the prepared fluids. If MF IV is dispensable it is shown that setups can be found to dispense all Newtonian IVD reagents, although all three fluid properties are high. The PDS dispenses MF IV without changing the parameter setup. The TPS passes the target volume with the second parameter setup (Fig. 6e and f).

Step 4: evaluation for dosing of non-Newtonian fluids by dispensing MF V

Pseudoplastic fluids feature a shear-rate-dependent viscosity, which leads to different flow profiles within a fluid channel. This can influence the dispensed volumes. Therefore, the dispensing systems should also be evaluated for non-Newtonian fluids by dispensing MF V. The PDS can still be operated with the parameter setup 1 and is not influenced by non-Newtonian

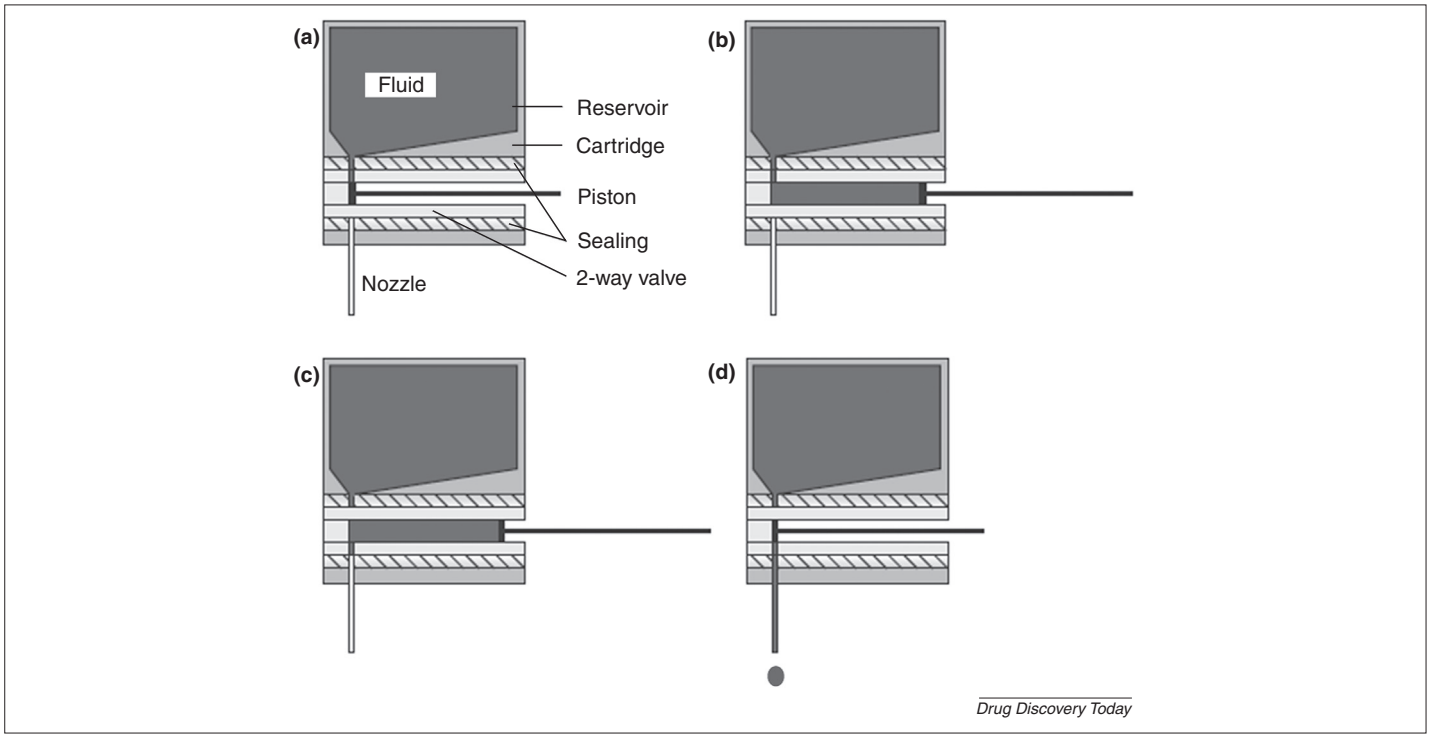

FIGURE 5

Working principle of the cartridge for dispensing a fluid. **(a)** Home position, **(b)** fluid intake by piston movement, **(c)** valve switching to nozzle and **(d)** fluid ejection by piston movement (European patent application EP12167108.5).

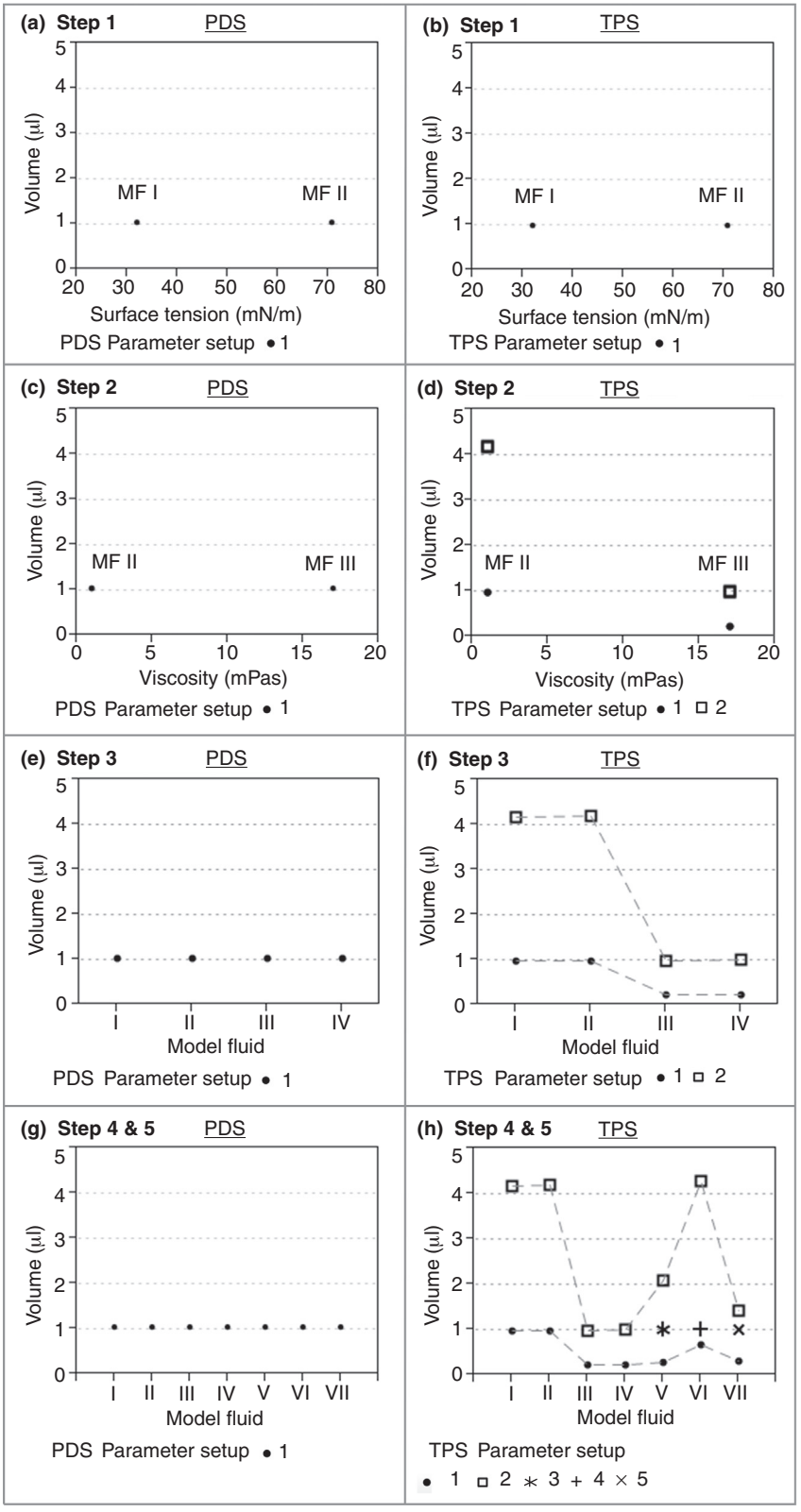

FIGURE 6

Dispenser evaluation for *in-vitro* diagnostic (IVD) applications in five steps. Left side: positive displacement system (PDS). Right side: time–pressure-dependent system (TPS). If different parameter settings are used to reach the target volume of 1 µl they are presented with altered symbols.

TABLE 2

Components and rheological properties of a selection of IVD reagents at 20°C

Fluid	Components	Density (kg/m^3)	Viscosity (mPas)	Surface tension (mN/m)
IVD reagents similar to model fluid I		998	1.0	71
105	Phosphate buffer 92 mmol/l, potassium hexacyanoferrate (III) 2.4 mmol/l	1022	1.0	71
141	Latex particles coated with monoclonal antihuman D-dimer antibodies (mouse) 0.15%	998	1.0	71
476	Sodium hydroxide 0.8 mol/l	1001	1.0	74
526	Fluorescein-labeled amikazin derivative in buffer, with stabilizer and preservative	1006	1.1	71
IVD reagents similar to model fluid II		998	1.0	32
111	CMV-AG~$Ru(bpy)_3^{2+}$, CMV-specific antigen (recombinant, *Escherichia coli*) labeled with ruthenium complex >400 μg/l, MES buffer 50 mmol/l, preservative	1008	1.1	31
487	Anticarbamazepine sheep serum in buffer, with stabilizer and preservative	1011	1.1	33
IVD reagents similar to model fluid IV		1169	16.9	31
CS[a]	Glycerol 63.6% (w/w), BSA 14.4 mg/ml, NaCl 22 mg/ml, Mannit 25.9 mg/ml	1185	16.4	55.3
IVD reagents similar to model fluid V		1005	8.6–13.4	67
73	Buffer solution, PVP, PEG 8000, 0.09% sodium azide	1024	8.4–14.8	67.8
380	Buffer solution, PVP, PEG 8000, 0.09% sodium azide	1024	8.4–14.4	67.5
IVD reagents similar to model fluid VI		1078	2.8	39
149	Antidigitoxin monoclonal antibody (mouse) in buffer with preservative	1060	2.8	66
259	Conjugated methadone derivate, buffer, bovine serum albumin, sodium azide 0.09%	1048	2.8	42
296	Phenobarbital conjugate, piperazine-N,N′-bis(ethanesulfonic acid)(PIPES)buffer, preservative, stabilizer	1045	2.9	34
299	Phenytoin conjugate, piperazine-N,N′-bis(ethanesulfonic acid)(PIPES)buffer, stabilizer, preservative	1015	2.7	43
535	Phosphate buffer 12.7 mmol/l, NaCl 0.13 mol/l, PEG 70 g/l, preservative	1035	2.9	31

Abbreviations: IVD, *in-vitro* diagnostic; PEG, polyethylene glycol; BSA, bovine serum albumin; PVP, polyvinylpyrrolidone; CS, complex solution; CMV, cytomegalovirus.
[a] This CS is used to represent the IVD reagents with the highest surface tension and viscosity. The corresponding IVD reagent is under development and, hence, the components are confidential.

fluids. The TPS needs a new parameter setup 3 to reach the target volume. The parameter setups 1 and 2 reach smaller and higher values than 1 μl, as is expected for a viscosity-dependent system. The dispensing results are shown in Fig. 6g and h.

Step 5: evaluation with different detergents

Up to now the dependencies on the fluid properties are specified. The yet to be tested MFs contain Sympatens® as the detergent. Detergents with other functional groups could show different behaviors regarding orifice wetting or sealing leakage. The MFs VI and VII include other organic components such as fatty acids and esters. If both are tested trouble-free, the system is evaluated for commonly used detergents and the risk that the system performance is influenced by other detergents is low. These two additional MFs can also be used to find the limit of the dispenser; for example, if one of the high viscous MFs (III and IV) are not dispensable. The PDS still requires only one parameter setup for all fluids (Fig. 6g), whereas the TPS needs two additional setups to dispense fluid VI and VII. Fig. 6h shows the results for the TPS system with the different setups and the volume changes. Moreover, for the TPS setups 1 and 2 are used to present the volume changes for all MFs, if the setup remains unchanged for all fluids.

Discussion and concluding remarks

The measured values of density, viscosity and surface tension show the distribution and the extreme values of the investigated IVD reagents. The range of the density is between 997 and 1192 kg/m^3 at 20°C, the viscosity is between 0.87 and 15.6 mPas at 20°C and the surface tension is between 26.2 and 77.8 mN/m at room temperature. These data are the basis for the development of MFs, representing the rheological properties (ρ, η and σ) and hence the principle dispensing behavior of the IVD reagents. To display the position of the fluids in the landscape of the IVD reagents, a viscosity–surface-tension plot is presented in Fig. 2. The fluids are located almost at the corners of the whole landscape, plus one fluid almost in the middle, one in the highest distribution and one representing the non-Newtonian fluids. Experiments with the TPS approve the theoretical assumption that complex IVD reagents are dispensable with the same results as simple MFs, if both have identical densities and viscosities (Fig. 4). Hence, to evaluate the suitability of a dispensing system for IVD applications there is no need to test with many different reagents.

This work presents a simple and time-saving method for dispenser evaluation. With five steps and seven MFs the dispenser is characterized regarding to its dependency on the fluid properties (ρ, η, σ) and the ability to dispense the parameter range of IVD reagents in a contact-free manner. The procedure is shown for two dispensing systems. Both systems can dispense all MFs, including Newtonian and non-Newtonian fluids. The volumes of the PDS are independent of the fluid properties, so that the system requires only one setup for all MFs, whereas the TPS depends on fluid viscosity and/or density leading to five different setups to obtain the target volume. The MFs can also be used for further characterization of a dispensing system. To evaluate the reproducibility, precision and accuracy, the procedure described by Bammesberger *et al.* [12] is recommended. Also, a dispenser evaluation using a design of experiments is possible to investigate quadratic dependencies or interactions between the fluid properties and the dispensed volumes, because the MFs I–IV are positioned at the edges and MF VII at the center point of the IVD reagent landscape. In conclusion, the presented work supports the evaluation of contact-free dispensing systems for IVD applications and offers a standardized and straightforward method to reduce cost and time.

Conflicts of interest

The materials used for this work were provided by the Roche Diagnostics GmbH. The authors are

solely responsible for conducting and analyzing the experiments.

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