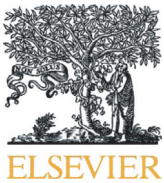




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# Migrating a lab-developed MERS-CoV real-time PCR to 3 “Sample to Result” systems: experiences on optimization and validation

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## ABSTRACT

The goal of the study was to adapt our Middle East respiratory syndrome coronavirus (MERS-CoV) lab-developed test (LDT) to 3 “Sample to Result” (S2R) systems: BD MAX (BD), ELITE InGenius (ELITechGroup), and ARIES (Luminex).

The BD MAX and InGenius system allowed use of lab-developed primers and TaqMan probes, while ARIES required conversion to MultiCode primers for melting curve analysis. Each device required ≤1 day of training and assay optimization. No discordant results were noted after analysis of 32 External Quality Control (EQC) samples. On a 10-fold dilution series of a MERS-CoV-positive EQC sample, InGenius obtained the highest detection rate. Laboratory technicians rated the ARIES as the user-friendliest. It also required the least hands-on time. BD MAX had the lowest turnaround time and highest throughput.

While each device had distinguishing system properties with associated (dis)advantages, the 3 S2R systems were comparable in terms of assay development and validation.

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## 1. Introduction

In September 2012, a new Coronavirus (CoV) was identified in patients whom had traveled to or resided in Saudi Arabia and suffered from acute respiratory distress with acute kidney injury (van Boheemen et al., 2012; Zaki et al., 2012). Due to its geographic predilection, the novel CoV was named Middle East respiratory syndrome coronavirus (MERS-CoV) (de Groot et al., 2013; van Boheemen et al., 2012; Zaki et al., 2012). Although limited in number, patients have also been reported in North Africa, Europe, Asia, and North America after returning from the Arabian Peninsula or after having contact with infected individuals. MERS-CoV is defined as a zoonotic disease as it is transmitted to humans through dromedary camels. Human-to-human transmission

is observed after close contact with infected patients (e.g., in healthcare settings) (Azhar et al., 2014).

WHO criteria for a laboratory confirmed MERS-CoV infection require detection of viral nucleic acid or antibodies (acute and convalescent samples) (World Health Organization (WHO), 2015). For viral nucleic acid detection, diagnostic real-time reverse-transcription polymerase chain reaction (RT-PCR) assays were developed for qualitative and quantitative detection of MERS-CoV in lower respiratory tract specimens (highest sensitivity), upper respiratory tract specimens, and/or serum (Corman et al., 2012; Lu et al., 2014; World Health Organization (WHO), 2015). The ability to rapidly detect MERS-CoV in clinical specimens is important for the timely initiation of treatment and isolation measures (Azhar et al., 2014; Corman et al., 2012; de Groot et al., 2013; Lu et al., 2014; van Boheemen et al., 2012; World Health Organization (WHO), 2015; Zaki et al., 2012).

“Sample to Result” (S2R) systems have revolutionized molecular diagnostics by automating nuclear extraction, amplification, and analysis inside 1 device (Beal et al., 2016). These systems provide fast results and can be operated in after-hour settings by laboratory technicians with limited molecular diagnostics experience (Beal et al., 2016). As the demand for urgent MERS-CoV PCR analyses increased in our center, especially after the yearly Hajj pilgrimage, we initiated this study to transfer our MERS-CoV lab-developed test (LDT) to an S2R system. Three devices were selected, evaluated, and compared in terms of hands-on experience, assay validation, and system properties.

**Abbreviations:** CoV, Coronavirus; EQC, External Quality Control; LDT, Lab-developed test; MERS, Middle East respiratory syndrome; MERS-CoV, Middle East respiratory syndrome coronavirus; PDV, Phocine Distemper Virus; RT-PCR, Real-time reverse-transcription polymerase chain reaction; S2R, Sample to Result; SPC, Specimen Processing Control; UTM, Universal Transport Medium.

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## 2. Materials and methods

### 2.1. MERS-CoV LDT

Total nucleic acid (TNA) is extracted from nasopharyngeal swabs in Universal Transport Medium (UTM, COPAN, Brescia, Italy) using NucliSens extraction on easyMAG (BioMérieux, Lyon, France). Ten microliters of Phocine Distemper Virus (PDV) internal control (IC) (Clancy et al., 2008) (PDV stock kindly supplied by Groningen Medical Center, Groningen, The Netherlands) is added to 200  $\mu$ L UTM sample to extract and elute 110  $\mu$ L of TNA. Five microliters of TNA extract is mixed with 1  $\times$  TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Waltham, MA), 0.5  $\mu$ M of each primer, and 0.2  $\mu$ M of each probe in a total volume of 20  $\mu$ L. The MERS-CoV Fast-PCR targets 3 genes using previously described primers and probes: MERS-CoV envelope gene *UpE* (Corman et al., 2012), MERS-CoV nucleocapsid gene *N* (Lu et al., 2014), and PDV hemagglutinin gene *H* (Clancy et al., 2008). Only the fluorescent dye combination for the MERS *N* gene probe was changed: Cy5-BHQ3. PCR amplification is performed on QuantStudio Dx (Thermo Fisher Scientific) using the following PCR temperature profile: 10 min 50 °C and 20 s 95 °C followed by 45 cycles of 3 s 95 °C and 30 s 60 °C. After the run, amplification plots are analyzed and interpreted using QuantStudio Test Development Software (version 1.0, Thermo Fisher Scientific). The presence of MERS-CoV in the sample is defined by amplification of *UpE* and/or *N* with the PDV IC.

Specificity of our LDT on QuantStudio Dx was validated using External Quality Control (EQC) samples (MERS-CoV-positive versus -negative samples), high-titer cell cultures (positive for hCoV 229E, NL63, OC43, or SARS), and clinical samples (positive for hCoV 229E, NL63, OC43, or HKU-1). Our LDT proved specific for MERS-CoV, and no cross-reactions were detected.

### 2.2. S2R conversion and evaluation

The assay was adapted to 3 S2R systems: BD MAX (Felder et al., 2014) (BD, Franklin Lakes, NJ), ELITE InGenius (ELITechGroup, Puteaux, France), and ARIES (Johnson et al., 2004; Juretschko et al., 2017; Sherrill et al., 2004) (Luminex, Austin, TX). A total of 200  $\mu$ L of input sample volume was used for extraction, PCR amplification, and result interpretation. Whenever system properties allowed, 10  $\mu$ L of the lab-developed PDV IC was added to the primary sample before extraction.

Comparison and evaluation of the 3 devices were based on 3 elements; required extraction and PCR optimization, MERS-CoV assay validation, and hands-on system experience. Validation of the MERS-CoV assay included determination of accuracy, specificity, cross-contamination, and cross-reaction through analysis of 32 EQC samples (QCMD/Instand): 16 MERS-CoV positive, 8 OC43-CoV positive, 1 NL63-CoV positive, and 7 CoV negative. The detection rate was evaluated by analysis of 5 dilutions in a 10-fold dilution series of a strong positive MERS-CoV EQC sample (Ct value Quantstudio  $\approx$  22). UTM was used as the dilution buffer. Linearity and analysis efficiency (extraction + PCR) were calculated from the Ct values obtained on the 10-fold dilution series. Lab-developed goals for linearity and analysis efficiency were set at  $R^2 > 0.950$  and  $> 80\%$ , respectively.

The hands-on time was determined once by the same operator and was defined as the time required to perform the following steps on 4 different samples: sample preparation, device loading, and device clean-up. Based on the total analysis time (=hands-on + on-board extraction + PCR time) and device sample capacity, the maximum throughput in a 1-day shift of 7.5 h was calculated. Three laboratory technicians without experience in molecular diagnostics were asked to test and grade the ease of use of all devices with “Very easy,” “Easy,” “Moderate,” “Difficult,” or “Very difficult.” Results were combined if there were discordances (e.g., “Easy/moderate”). Any additional comments on the required training, device setup (including sample and

reagent loading), software interface, quality of the generated report, and maintenance (including clean-up) were noted.

## 3. Results

### 3.1. Required extraction and PCR optimization

The proposed sample input volume of 200  $\mu$ L was used on each system (Table 1). A volume of 10  $\mu$ L in-house PDV IC was used as an extraction and amplification control on the BD MAX and InGenius. On ARIES, a Luminex-patented extraction and amplification control included in the reaction cassette was used (Table 1).

Luminex ARIES uses no probes but specific *MultiCode* primers (Johnson et al., 2004; Sherrill et al., 2004). Luminex provided *MultiCode* primers for the MERS-CoV *upE* and *N* targets (no alterations to our original sequence) together with primers for the ARIES-specific internal control. Primer concentrations of 0.2  $\mu$ M were recommended by Luminex, but in our experience, 0.4  $\mu$ M resulted in an improved balance between weak positive signals and aspecific amplification products. Therefore, 0.4  $\mu$ M was used in all subsequent experiments. The general ARIES temperature profile yielded satisfactory results: 7 min 50 °C and 2 min 95 °C followed by 45 cycles of 5 s 95 °C and 7 s 58 °C. Afterwards, melting curve analysis was performed from 60 °C to 95 °C. On the BD MAX and InGenius, unmodified primers/probes with the Fast Virus 1-Step Master Mix and lab-developed PCR temperature profile yielded satisfactory results. Concentrations of reagents remained unchanged. As the BD MAX required sufficient time for fluorometric analysis of all 24 reaction chambers in the PCR cartridge, the annealing/extension time was adjusted from 30 s to the maximum of 24.9 s.

### 3.2. MERS-CoV assay validation

Results of the validation experiments in comparison to the LDT are displayed in Table 2. No discordant or false-positive results were noted after analysis of 32 EQC samples. There was no cross-contamination. The InGenius obtained the highest detection rate by yielding positive results for 5/5 dilutions for *upE* and *N*. Goals for linearity and analysis efficiency were reached with both targets on all 3 systems.

The ARIES system had a lower detection rate on the dilution series and yielded positive results for 3/5 and 4/5 dilutions for *upE* and *N*, respectively (Table 2). Some of the MERS-CoV-negative EQC samples also resulted in amplification plots with high Ct values. These amplifications proved aspecific based on the melting curve and  $T_m$  interval. As they fell outside of the prespecified  $T_m$  intervals for *upE* and *N*, these plots were called negative by the automatic software interpretation.

### 3.3. Hands-on system evaluation

For all 3 devices, 1-day on-site training proved to be enough to cover all major aspects of using system-specific assays (Table 3). The flow of sample preparation, device loading, TNA extraction, PCR, and clean-up are displayed in Fig. 1.

On the ARIES, the *MultiCode* primers are pipetted manually into conical tubes containing lyophilized *MultiCode Ready Mix*. Each tube is then snapped into an analysis cassette. After pipetting of the primary sample, the cassette is placed into a magazine and loaded onto the device. The sample is immediately subjected to extraction, RT-PCR, and melting curve analysis. Clean-up consists of removing the cassettes from the device.

The BD MAX uses *Sample Buffer tubes* containing 750  $\mu$ L lysis buffer in which primary sample and IC are pipetted manually. After barcode scanning, tubes are placed into a rack containing 1 *Extraction Reagent Strip* per sample. The PCR mix (= primers, probes, and mastermix) is pipetted manually into a conical snap-in tube inserted into an *Extraction Reagent Strip*. The rack is then placed in the device, and after startup,

**Table 1**  
Required extraction/PCR optimization for the described MERS-CoV LDT.

	ARIES	BD MAX	InGenius
Required PCR optimization (time)	≤1 day	≤1 day	≤1 day
Sample volume (μL, range)	200 (200–400)	200 (100–750)	200 (200 or 1000 <sup>a</sup> )
Extract volume (μL, range)	150 (not applicable)	12.5 (not applicable)	50 (50, 100, or 200)
PCR reaction volume (μL, range)	56 (not applicable)	4 (not applicable)	20 (20 to 50)
Extract volume used for PCR (μL)	50	2	5
Sample equivalent analyzed in PCR (μL)	66,6	32	20
Analysis method	Melting curve analysis <sup>b</sup>	Taqman hydrolysis	Taqman hydrolysis
Internal extraction and PCR control	ARIES-specific, in cassette	Lab-developed PDV, 10 μL, pipetted manually (+ SPC <sup>c</sup> )	Lab-developed PDV, 10 μL, pipetted automatically
Primers	MultiCode converted, pipetted manually on top of mastermix in snap-on tube, 0.4 μM each	Unmodified lab-developed, mixed with mastermix, 0.5 μM each	Unmodified lab-developed, mixed with mastermix, 0.5 μM each
Probes	Not applicable	Unmodified lab-developed, mixed with mastermix, 0.2 μM each	Unmodified lab-developed, mixed with mastermix, 0.2 μM each
Mastermix	ARIES MultiCode Ready Mix, lyophilized, present in snap-on tube	1 × Taqman Fast Virus 1-Step, 12.5 μL, pipetted manually in snap-in tube	1 × Taqman Fast Virus 1-Step, 15 μL, pipetted automatically
PCR optimization	- Generic ARIES PCR - MultiCode primer conversion - Primer concentrations	Slightly modified lab-developed PCR with adjusted annealing and extension times	Identical to lab-developed PCR

PDV = Phocine Distemper Virus; SPC = Specimen Processing Control; TNA = Total nucleic acid.

<sup>a</sup> InGenius extraction kits for 1000-μL sample volumes are under development at the time of writing.

<sup>b</sup> ARIES Exo + Ready Mix supporting TaqMan probes is under development at the time of writing.

<sup>c</sup> The *Sample Buffer Tube* of the BD MAX extraction kit also contained a *Specimen Processing Control* (SPC), which can be used as an internal control but was not utilized in our study.

the BD MAX automatically pipets the lysed sample into the *Extraction Reagent Strip* for TNA extraction. The TNA extract is subsequently added to the PCR mix and transferred to the PCR card. RT-PCR is performed in dedicated PCR cards (24 samples/card) with a built-in microfluidics system. Clean-up consists of removing the tubes and strips from the rack and, at the end of the day, cleaning up the interior surface of the device.

The InGenius can accommodate primary samples pipetted into sample tubes (= extraction and PCR mode) or TNA extracts pipetted into cryovials (= PCR only mode). The InGenius is the only system where the TNA eluate can be stored and used for multiple PCRs in subsequent runs (Table 3). The device uses disposables (sample tubes, extract cryovials, extraction strips, PCR cassettes, filter tips) that must be loaded onto the device before analysis. IC and PCR mix (= primers, probes, and mastermix) are placed into a cooled reagent block. After loading, the system starts the extraction (if needed) and PCR. Clean-up consists of removing all disposables, storing remaining IC and PCR mix, storing remaining TNA extract (if needed), and cleaning of interior surfaces.

ARIES was classified as user-friendliest due to its analysis cassette, which required the least hands-on time and manipulation (Table 3). The technicians judged that the manual pipetting steps on the ARIES

and BD MAX were the most error-prone. With InGenius, although the system guides the user through reagent and consumable loading, we noticed that it required some training and handling experience to avoid system crashes (e.g., by misalignment of disposables).

ARIES required the least hands-on time. The BD MAX had the shortest analysis time and, in combination with its capability of analyzing 2 × 12 samples in 1 run, the highest throughput in a 1-day shift: 120 samples in 5 runs (Table 3). Different assays can be loaded and analyzed in the same run on all systems if specific conditions are met (Table 3). On the BD MAX and InGenius systems, PCR settings can differ between lanes as the 24 chambers in the BD MAX PCR card and 12 InGenius thermocyclers are individually controlled. Besides the ability to run different PCR assays in 1 run, the instrument “down-time” between analyses is important for urgent sample testing. For the InGenius system, this “down-time” equals the onboard extraction and PCR time (Table 3). For BD MAX, it is limited to the extraction time as new samples can be loaded during PCR. However, the 2 independent extraction trays on the BD MAX system cannot be accessed if extraction is already ongoing in 1 of them. The ARIES system has 2 independent loading trays; if 1 module is running, the other can be used without restrictions.

**Table 2**  
MERS-CoV LDT validation.

	QuantStudio Dx	ARIES	BD MAX	InGenius
Accuracy <sup>a</sup>	No discordance	No discordance	No discordance	No discordance
Specificity <sup>a</sup>	No false positives	No false positives	No false positives	No false positives
Cross-reaction <sup>a</sup>	None	None	None	None
Cross-contamination <sup>b</sup>	None	None	None	None
MERS-CoV UpE:	Detected: 5/5 dilutions	Detected: 3/5 dilutions	Detected: 4/5 dilutions	Detected: 5/5 dilutions
Detection rate	Ct range <sup>c</sup> : 23.7–35.3	Ct range <sup>c</sup> : 30.0–37.1	Ct range <sup>c</sup> : 23.4–32.7	Ct range <sup>c</sup> : 23.5–37.6
/ Linearity <sup>d</sup>	R <sup>2</sup> : 0.988	R <sup>2</sup> = 1.000	R <sup>2</sup> : 0.969	R <sup>2</sup> : 0.996
	118.3% efficiency	91.2% efficiency	116% efficiency	92.7% efficiency
MERS-CoV N:	Detected: 5/5 dilutions	Detected: 4/5 dilutions	Detected: 4/5 dilutions	Detected: 5/5 dilutions
Detection rate	Ct range <sup>c</sup> : 24.2–36.2	Ct range <sup>c</sup> : 29.1–39.5	Ct range <sup>c</sup> : 24.4–32.9	Ct range <sup>c</sup> : 22.9–35.2
/ Linearity <sup>d</sup>	R <sup>2</sup> : 0.967	R <sup>2</sup> = 0.998	R <sup>2</sup> : 0.981	R <sup>2</sup> : 0.999
	105.4% efficiency	92.3% efficiency	131% efficiency	109.7% efficiency

<sup>a</sup> Analysis of 32 EQC samples: 16 MERS-CoV positive, 8 OC43-CoV positive, 1 NL63-CoV positive, and 7 CoV negative.

<sup>b</sup> Determined by analysis of a negative sample adjacent to a positive sample.

<sup>c</sup> MultiCode Ct values are typically higher than TaqMan Ct values (Voermans et al., 2016).

<sup>d</sup> Determined on a 10-fold dilution series (5 dilutions) of a strong positive MERS-CoV EQC sample (Ct value Quantstudio ≈ 22).

**Table 3**  
Hands-on system evaluation.

	ARIES	BD MAX	InGenius
Required on-site training	1 day	1 day	1 day
Possibility to load primary samples	No, manual pipetting required	No, manual pipetting required	Yes
Storage of TNA extract	Not available	Not available	Available
Ease of use	Very easy	Easy	Easy/moderate
Most challenging step	Manual pipetting of primers	Manual pipetting of mastermix	Loading of disposables
Total hands-on time for 4 samples	6 min 5 s	12 min 25 s	12 min 0 s
- Sample preparation	4 min 25 s	4 min 35 s	1 min 20 s
- Device loading	1 min 10 s	5 min 40 s	6 min 50 s
- Device clean-up	0 min 30 s	2 min 10 s	3 min 50 s
On-board extraction and PCR time	1 h 54 min	1 h 22 min	2 h 12 min
Total analysis time	2 h 0 min 5 s	1 h 34 min 25 s	2 h 24 min 0 s
Reagent cost per sample <sup>a</sup>	Contact company	25.7 €	12.1 €
Maximum throughput in a 1-day shift (7.5 h)	- 2 × 6 samples/run - ± 2 h/run - 36 samples in 3 runs	- 2 × 12 samples/run - ± 1.5 h/run - 120 samples in 5 runs	- 12 samples/run - ± 2.5 h/run - 36 samples in 3 runs
Different assays in the same run	Yes, same PCR profile	Yes, same extraction kit	Yes, same extract elution volume
Number of optical channels for multiplexing	5 optical channels + melting curve	5 optical channels + melting curve	6 optical channels + melting curve
Software package and location	SYNCT: separate PC and device	BD MAX: PC next to device	InGenius: device
Automatic result interpretation	Programmable interpretation. Ct value limits and T <sub>m</sub> intervals.	Programmable interpretation. Ct value limits.	Programmable interpretation. Ct value limits.
Onboard reagent traceability	Extraction cassette	Sample Buffer Tube Extraction cassette	Extraction cassette User-defined reagents
LIS communication <sup>b</sup>	2-way connection	2-way connection	2-way connection

<sup>a</sup> If available, based on the list price (VAT excluded) at the time of writing.

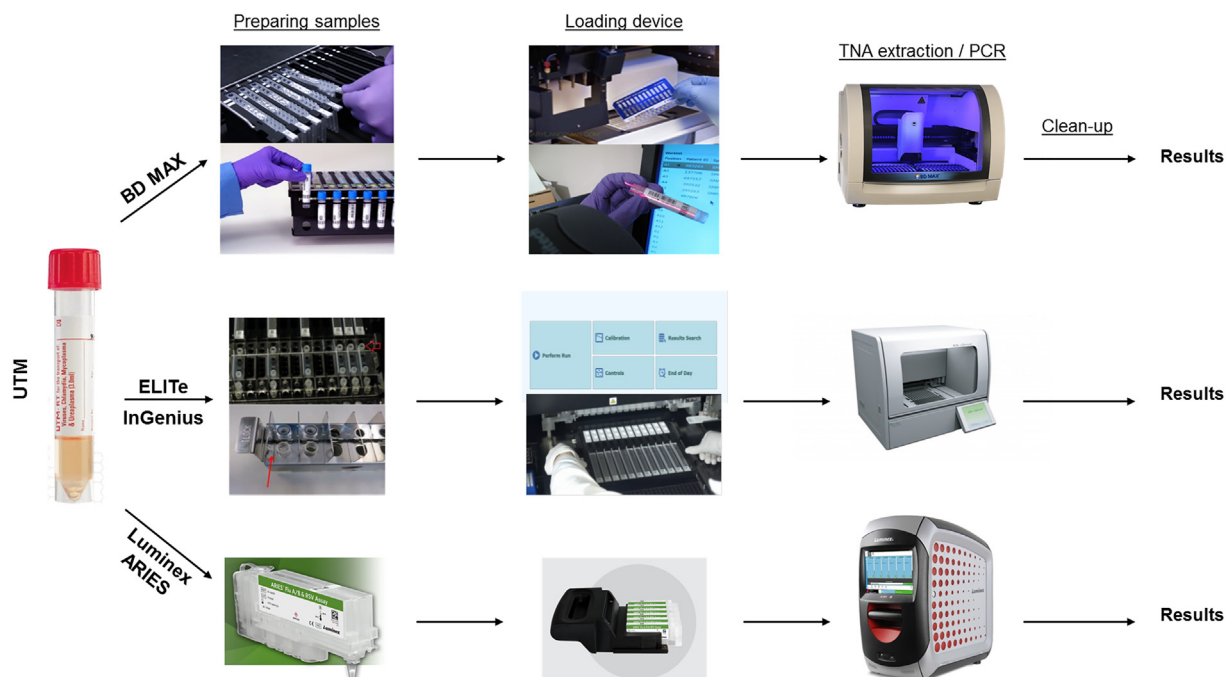
<sup>b</sup> Was not evaluated during this study.

Information about the supplied software packages is displayed in Table 3. On ARIES, an *open mode* PCR assay must be set up from the SYNCT software on a remote desktop after which the protocol is transferred to the instrument using USB (only needs to be done once). On the InGenius, the supplier must create a new *open mode* PCR protocol, define the assay name, and register PCR reagent volumes on the device. Afterwards, the user can modify all protocol settings. ARIES and BD MAX software allowed modification of all protocol settings without any supplier input. Lot numbers of the system-specific reagents are registered in all software packages (Table 3). On the InGenius, the user can also add lab-developed PCR mix and IC lot numbers to the registry. Although this was not evaluated in our study, all devices can be programmed for

automatic interpretation of PCR results using software models and Ct value limits (Table 3). ARIES also accounts for dye/target T<sub>m</sub> intervals. Determination of specific T<sub>m</sub> intervals in the SYNCT software required us to test a number of additional positive/negative samples.

#### 4. Discussion

The ability to rapidly detect or exclude MERS-CoV infections in clinical specimens is important for diagnosis confirmation or termination of isolation measures, respectively (Azhar et al., 2014; Corman et al., 2012; de Groot et al., 2013; Lu et al., 2014; van Boheemen et al., 2012; World Health Organization (WHO), 2015; Zaki et al., 2012). Automated S2R



**Fig. 1.** Graphical representation of the sample preparation, device loading, TNA extraction, PCR, and clean-up for the BD MAX, ELITE InGenius, and Luminex ARIES. Copyright statement: Fig. 1 contains several images derived from device product manuals. Permission was obtained from BD, ELITEch, and Luminex to include these images in our manuscript.

systems provide time and labor savings while delivering simpler workflows through elimination of separate nucleic acid extraction and hands-on steps (Beal et al., 2016). In this study, we migrated our MERS-CoV LDT to 3 *open mode* S2R systems and compared their system properties.

As the Luminex ARIES works through melting curve analysis and Tm intervals, no probes were needed (Johnson et al., 2004; Sherrill et al., 2004). This means that primers might have to be redesigned to exclude off-target amplicons with the same Tm. Our MERS-CoV primers only required addition of a fluorescent-labeled isoC base at the 5' end of the forward primers for conversion to the *MultiCode* format. As our PDV IC would have required an extra pipetting step and the development of an additional *MultiCode* primer, we decided to use the built-in RNA IC of the ARIES cassettes. It remained unclear whether the lower detection rate obtained with ARIES was due to *MultiCode* primer conversion, melting curve analysis, or limited optimization runs. It is however important to realize that due to differences in technology, *MultiCode* Ct values are typically higher than TaqMan Ct values (Voermans et al., 2016). Of interest, Luminex reported the release of a new ARIES mastermix and software update in the coming months to also utilize fluorescent labeled TaqMan probes (personal communication).

Device properties hugely influence the selection of a S2R device. First, available CE/IVD and company-developed tests are an important factor to consider. Second, the instrument “down-time” during analysis differs between devices and is important for urgent sample testing. Third, the ability to analyze several targets on the same sample is different for each system. The InGenius system has the advantage that a single extract (providing there is enough extract volume) can be used in 12 separate multiplex reactions (up to 6 optical channels + melting curve) in 1 run. For BD MAX, this is limited to 2 separate multiplex reactions (up to 5 optical channels + melting curve) and, for ARIES, to 1 multiplex reaction (5 optical channels + melting curve). Fourth, system-specific advantages need to be taken into account. InGenius is the only system where the remaining TNA extract can be recovered for storage or further analysis. InGenius is also the only system where the PCR volume and the added TNA volume can be increased to improve sensitivity. In comparison, BD MAX has the highest throughput and Luminex ARIES excels in ease of use. Finally, differences exist in the availability of quantitative assays. While this was not further evaluated during our study, InGenius allows definition of standard curve options for single tests/fluorescent dyes and sample type dependent conversion factors. A standard curve can also be defined on a single test/fluorescent dye on the BD MAX. Quantitative assays have only been developed in research settings for ARIES. At the time of writing, Luminex is developing a SYNCT software update for incorporating standard curves for quantitative analysis (personal communication).

Although our MERS-CoV PCR assay proved both sensitive and specific on all devices, there were several limitations to the study. First, as no MERS-CoV patients were diagnosed in our center, all data were derived from EQC samples which may not be representative for clinical samples during natural infection. EQC samples were not subjected to the same collection, handling, and storage conditions as clinical samples. In addition, only 16 MERS-CoV-positive, 8 OC43-CoV-positive, 1 NL63-CoV-positive and 7 CoV-negative EQC samples were tested, which limit statistical analysis and interpretation of the cross-reaction experiments. Nevertheless, the assays on the 3 systems use the same primers/probe sets and master mix (excluding Luminex ARIES) as our lab-developed PCR on QuantStudio Dx (Clancy et al., 2008; Corman et al., 2012; Lu et al., 2014). The specificity of this LDT was evaluated using high-titer cell cultures positive for hCoV 229E, NL63, OC43, and SARS. Clinical samples positive for other respiratory viruses, including hCoV 229E, NL63, OC43, and HKU-1, were all negative. Second, assay validation experiments were only performed once due to limited reagents and limited availability of the instruments to our laboratory. Therefore, statistically significant differences could not be determined. Especially the difference in detection rate needs to be interpreted with

caution as sample composition could influence the extraction efficiency of each device. The hands-on time was measured only once by 1 operator. While this gives an overall indication on the hand-on time required for each device, our analysis does not provide insight in the real-life hands-on time (e.g., different operators, trained versus untrained personnel, day-to-day variability). Third, intra- and inter-run reproducibility was not determined in our study due to limited reagents.

In conclusion, while each device had distinguishing system properties with associated advantages/disadvantages, migration of a MERS-CoV LDT to 3 S2R systems was comparable in terms of assay development and validation.

### Author contributions

GF and KB drafted the manuscript and conducted experiments. All authors participated in the conception and design of the study. MVR, VS, SD, and KL supervised the routine molecular diagnostics workflow of MERS-CoV samples. Each author has critically revised the final version of the manuscript and has read and approved the final manuscript.

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### Disclosure of potential conflicts of interest

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#### Conflicts of interest

The authors declare that they have no conflict of interest.

#### Compliance with ethical standards

Research involving human participants and/or animals: not applicable.

Informed consent: not applicable.

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