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# EVALUATION OF AN AUTOMATIC URINOMETER INCLUDING USE OF SILICONE OIL TO DECREASE BIOFILM FORMATION DUE TO PROTEINURIA, HEMOGLOBINURIA AND BACTERIAL GROWTH

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# EVALUATION OF AN AUTOMATIC URINOMETER INCLUDING USE OF SILICONE OIL TO DECREASE BIOFILM FORMATION DUE TO PROTEINURIA, HEMOGLOBINURIA AND BACTERIAL GROWTH

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

**Martin Slettengren, M.D.**

**Public defense on Thursday 14th of January 2021 at 1.30 PM**

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Due to COVID-19 it is strongly recommended to use ZOOM to follow the dissertation: https://ki-se.zoom.us/j/66174030955

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“Gräv ut slöhetens bomull ur förståndets öra, så att från de döde visdomsordet må tränga in i ditt öra”

– Shaikh Saadi

(*Persisk poet 1210–1292*)

# ABSTRACT

**Background:** A new capacitance-based automatic urinometer (AU) facilitates continuous urine output (UO) measurement, which may help to predict and diagnose acute kidney injury (AKI). To prevent mismeasurement due to bacterial, albumin or free hemoglobin biofilm, a water-soluble capsule with silicone oil has been integrated in the device.

**Aims:** To assess: the performance of a new capacitance-based AU in adult patients in a cardiothoracic intensive care unit (ICU) and compare it with a manual urinometer (MU) in regard of bias, precision, temporal deviation and to evaluate the staff's opinion of the AU (**Study I**); a modified capacitance-based AU in comparison with an MU regarding measuring bias among patients ≤10 kg in a pediatric intensive care unit and to evaluate the staff's opinion of the AU (**Study II**); whether a silicone oil-coated polypropylene plastic surface, as used in an AU, may reduce early microbial biofilm formation and to identify the silicone oil target; to compare polypropylene with polystyrene and low with medium viscosity silicone oil regarding the propensity to impede biofilm formation (**Study III**); if silicone oil added to the measuring chamber of the AU may prevent the rise in capacitance due to albumin or free hemoglobin biofilm, allowing the device to function for longer periods of time (**Study IV**).

**Methods: Study I-II** were prospective observational cohort studies, whereas **Study III-IV** were experimental prospective *in vitro* studies. **Study I**: 34 postoperative patients had their hourly UO registered with either an AU (n=220) or an MU (n=188), which were validated by cylinder measurements and analyzed using the Bland-Altman method. The temporal deviation of the MU measurements was recorded (n=108) and at the end, the nursing staff (n=28) evaluated the AU. **Study II**: The hourly diuresis was measured using either an AU (n=127) or an MU (n=83) in 12 children (weight ≤10 kg) and validation was carried out using a measuring cylinder. Thereafter, the nursing staff (n=18) evaluated the AU. **Study III**: Clear flat-bottomed wells of either polypropylene or polystyrene were pretreated with silicone oil of low or medium viscosity, after which a panel of microbes, including common uropathogenic bacteria and *Candida albicans,* were added. The plates were left for 3 days and the amount of biofilm formation was assessed using the crystal violet assay. **Study IV:** A solution of Ringer's acetate mixed with either albumin or free hemoglobin was run through an AU with either a water-soluble capsule with silicone oil (n=20) or not (n=20)

and the derived 400-500 capacitance measurements, respectively, were retrieved from the AU device and analyzed.

**Results: Study I**: The AU had a smaller mean bias (+1.9 mL) than the MU (+5.3 mL) ($p<0.0001$). Defined by their limits of agreements (±15.2 mL AU vs. ±16.6 mL MU, $p=0.11$), the measurement precision of the two urinometers were similar. The AU had inherently no temporal deviation, whereas the mean temporal deviation of the MU was ±7.4 minutes (±12.4%) ($p<0.0001$). The nursing staff rated the AU significantly higher than the MU in terms of user-friendliness, measuring reliability, efficacy and safety. **Study II**: The AU and the MU had a mean bias of −1.1 mL (CI, −0.6 to −1.5) and −0.6 mL (CI, ±0.0 to −1.2) respectively ($p=0.21$). The participating staff considered the AU significantly easier to learn, use and handle compared with the MU. **Study III**: Polypropylene plastic exhibited less biofilm growth than polystyrene. Silicone oil, irrespective of viscosity, significantly decreased biofilm formation by common uropathogenic bacteria, including ESBL-producing and multi-drug resistant strains, as well as *C. albicans*. *E. coli* curli fimbriae were established as the main focus of silicone oil. **Study IV**: The mean increase in capacitance with albumin 3 g/L group was 257±96 without and 105±32 with silicone oil, respectively, during 24 hours. After ten hours of registration, differences between the two albumin groups reached statistical significance. For the free hemoglobin groups (0.01 g/L), the mean increase in capacitance was 190±174 with silicone oil and 324±78 without. A significant difference between the free hemoglobin groups was seen after 20 hours and onwards.

**Conclusions:** For adult postoperative patients, the AU was non-inferior to the MU with regard to measuring precision and significantly better than the MU in terms of bias and temporal deviation (**Study I**); for children weighing ≤10 kg, the urinometers were comparable in performance (**Study II**); staff consistently appraised the AU significantly higher than the MU in terms of user-friendliness, reliability, safety and efficacy (**Study I** and **II**). Both low and medium viscosity silicone oil coating of a polypropylene surface decreased biofilm formation from common uropathogenic bacteria including *Candida albicans* and the biofilm-promoting factor curli fimbriae was identified as a plausible target (**Study III**); coating of the capacitance measurement membrane of the AU by albumin or free hemoglobin significantly disturbed the capacitance measurement capability of the AU, and this could be prevented by incorporating silicone oil in the device (**Study IV**).

# LIST OF SCIENTIFIC PAPERS

I. Eklund A, **Slettengren M**, van der Linden J. Performance and user evaluation of a novel capacitance-based automatic urinometer compared with a manual standard urinometer after elective cardiac surgery.
*Critical Care.* 2015;19:173.

II. **Slettengren M**, Wetterfall H, Eklund A, van der Linden J. A pilot evaluation of a capacitance-based automatic urinometer in a pediatric intensive care setting. *Pediatr Crit Care Med.* 2019;20(8):769-772.

III. **Slettengren M***, Mohanty S*, Kamolvit W, van der Linden J, Brauner A. Making medical devices safer: impact of plastic and silicone oil on microbial biofilm formation. *J Hosp Infect.* 2020;106:155-162. (* shared first author)

IV. **Slettengren M**, Linnros M, van der Linden J. Silicone oil decreases biofilm formation in a capacitance-based automatic urine measurement system.
*(In Manuscript)*

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AKI | Acute kidney injury |
| AKIN | Acute kidney injury network |
| AU | Automatic urinometer |
| Bsc | Bacterial cellulose synthase |
| CABG | Coronary artery bypass grafting |
| CFU | Colony forming units |
| Cm | Main capacitance sensor |
| CPB | Cardiopulmonary bypass |
| Csg | Curli specific gene |
| Cr | Reference capacitance sensor |
| ECMO | Extracorporeal membrane oxygenation |
| ESBL | Extended spectrum beta lactamase |
| fHb | Free hemoglobin |
| GFR | Glomerular filtration rate |
| ICU | Intensive care unit |
| KDIGO | Kidney Disease Improving Global Outcome |
| LB | Luria-Bertani |
| LOA | Limit of agreement |
| LOS | Length of stay |
| MEWS | Modified early warning score |
| MU | Manual urinometer |
| PDMS | Polydimethylsiloxane |
| RIFLE | Risk, Injury, Failure, Loss and End-stage renal disease |
| PP | Polypropylene plastic |
| RPM | Revolutions per minute |
| SD | Standard deviation |
| 10 SOV | 10 signs of vitality |
| UO | Urine output |
| YPD | Yeast-peptone-dextrose |

# 1 INTRODUCTION AND BACKGROUND

## 1.1 WHY REGISTER URINE OUTPUT

For hospitalized patients, particularly for those in the intensive care unit (ICU), essential physiological parameters, including arterial saturation, heart rate, blood pressure, temperature and respiration rate, are routinely recorded and displayed on the patient data monitoring system. The used measuring devices will warn the staff if the monitored values are not within the set reference range, whereupon, when necessary, they may help to reduce risk for organ failure and ultimately death.

Urine output (UO) is a vital part of the patient´s fluid turnover, representing the lion's share of fluid output and corresponds to approximately 1500 mL/day in a resting adult person. In contrast to fluid intake, which is commonly automatically registered via pumps and syringes, UO is virtually still exclusively recorded manually.

Measuring UO hourly may warn of acute kidney injury (AKI), which is not an unusual problem after major surgery and periods of hemodynamic instability (1, 2). AKI occurs in more than 55% of ICU patients (3) while cardiac surgery has been associated with up to 30% incidence of AKI (4). Ischemia, sepsis and toxic medication are considered the most frequent triggers of AKI in both children and adults (5). In one study, 25% of pediatric ICU patients with AKI died within 4 weeks, whereas the mortality rate in children without AKI was 2.7% during the same period (6).

UO is included alongside creatinine and glomerular filtration rate (GFR) in the internationally used systems RIFLE, AKIN and KDIGO to define AKI (**Figure 1**) (7, 8).

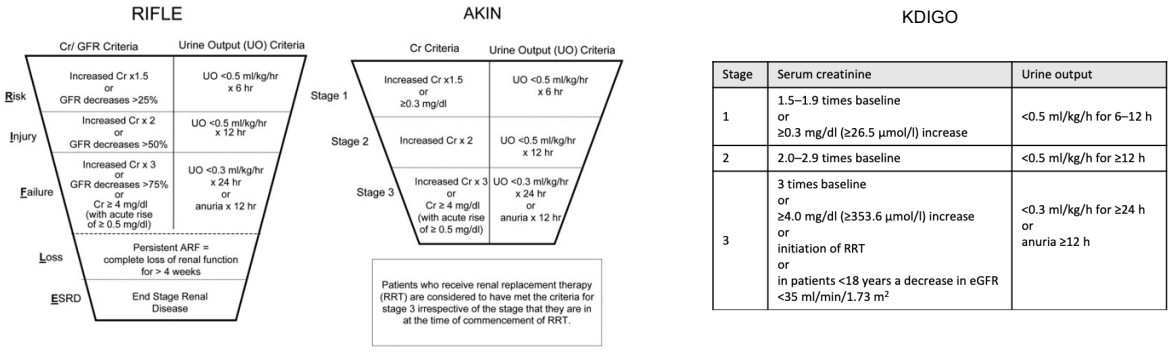



**Figure 1.** Internationally used systems, RIFLE (Risk Injury Failure Loss and End-stage renal disease, left), AKIN (Acute Kidney Injury Network, middle), and KDIGO (Kidney Disease Improving Global Outcome, right) to define AKI (7). (Reprinted with permission from BMC Springer Nature and Karger Publishers).

Serum creatinine has a low sensitivity for AKI as almost 50% of the GFR must be lost before a change in serum creatinine may be detected (9). Although the importance of UO as a solitary parameter to reflect kidney function has been debated, continuous monitoring of UO in addition to renal laboratory parameters may help to identify AKI (10-12) and be related to outcome. In a prospective study by Macedo et al. including 317 ICU patients, UO was found to be both an early and sensitive marker for AKI and correlated to an increased mortality rate in ICU patients (13). In another study by Kellum et al. (14), isolated oliguria in stage 2 and 3 AKI was associated with a decreased 1-year survival. Moreover, prediction of both short- and long-term risk of death or renal replacement therapy was greatest when both the serum creatinine and the UO criteria were met together.

UO is probably a more sensitive but less specific criterion that needs to be addressed in context with the patient's clinical status and medical history. In a study by Prowle et al., it was found that solitary oliguria, without taking other parameters into consideration, was only an average predictor of AKI. However, if the patient was hemodynamically unstable or in need of incrementing vasopressor support, oliguria was a clinically helpful indicator to identify patients in danger of developing AKI (15). Although a low UO is usually associated with pending or manifest AKI, UO might in some cases of AKI also increase, e.g. in acute interstitial nephritis. Furthermore, the patient's fluid status will influence the UO. Hypovolemia for instance will trigger a low UO without the absolute need of concomitant kidney injury (9).

UO during cardiopulmonary bypass (CPB) has been shown to be independently associated with the risk for developing AKI (16) and oliguria post cardiac surgery in association with a positive fluid balance has been shown to better predict longer hospital length of stay (LOS) than oliguria alone (17).

Interestingly, hourly UO is one of six simple parameters (heart rate, systolic blood pressure, respiratory rate, temperature, state of consciousness, UO) that are included in the modified early waring score (MEWS) score, which is used to identify deteriorating patients on hospital wards who may need intensive care (**Table 1**) (18). It is also one of 10 vital parameters included in another early warning system called 10 signs of vitality (10 SOV) (**Table 2**) (18). Several other therapeutic protocols use UO to evaluate patients' response to treatment, e.g. resuscitation in septic shock (19, 20) and management of burn patients (21, 22).

**Table 1**. Urine output is included in the MEWS (modified early warning score), which is used to identify deteriorating patients on hospital wards who may need intensive care (18).

| **Score** | 3 | 2 | 1 | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|---|---|
| Respiratory rate, per min | – | ≤8 | – | 9-14 | 15-20 | 21-29 | >29 |
| Heart rate, per min | – | ≤40 | 41-50 | 51-100 | 101-110 | 111-129 | >129 |
| Systolic blood pressure, mm Hg | ≤70 | 71-80 | 81-100 | 101-199 | – | ≥200 | – |
| Urine output, $mL \cdot kg^{-1} \cdot h^{-1}$ | Nil | <0.5 | – | – | – | – | – |
| Temperature, °C | – | ≤35 | 35.1-36 | 36.1-38 | 38.1-38.5 | ≥38.6 | – |
| Neurological | – | – | – | A | V | P | U |

The score for each parameter is recorded at the time that observations are taken. If the total is four or more then the ward doctor is informed. A = alert; V = reacting to voice; P = reacting to pain; U = unresponsive

**Table 2**. Urine output is one of 10 vital parameters included in another early warning system called 10 SOV (10 Signs Of Vitality) (18).

| **Sign** | **Abnormal range** | |
|---|---|---|
| Temperature | ≥38°C | Unweighted measures |
| Pulse | <50 or >100/min | |
| Pain | New or significant | |
| Respiratory rate | <6 or >20/min | Weighted measures |
| $SaO_2$ | <90% or increasing $O_2$ requirement | |
| Blood pressure | Mean arterial pressure<60 mm Hg or systolic blood pressure <90 mm Hg | |
| Level of consciousness | Agitation, anxiety, apathy, lethargy, stupor, or coma | |
| Urine output | <30 mL/h or <100 mL/4 h excluding renal failure | |
| Capillary refill | >3 s | |
| Temperature | <36°C | |
| Lactic acid and metabolic acidosis | >2 meq/L or base deficit ≥5 meq/L | |

Any one abnormality of the above signs triggers an assessment by the bedside nurse of all 10 signs. Presence of ≥2 weighted abnormalities suggests significant hypoxia or hypoperfusion, thereby triggering a mobilization of the Rapid Response Team.

## 1.2 MANUAL REGISTRATION OF URINE OUTPUT

UO is manually registered recurrently (usually hourly) by the nursing staff in wards and in ICUs. Manual measuring is based on visual evaluation of urinometers and collection bags and data are documented manually. UO of ICU patients is gathered in a graded container that usually has a maximal volume of approximately 500 mL. The container is attached to a plastic bag that has a volume of a 1500-3000 mL.

### 1.2.1 Disadvantages with manual registration of urine output

Manual registration of UO requires a considerable amount of time and effort by the working staff. The whole process of recording UO manually requires up to two minutes (23). In an ICU with 12 patients, this translates to 24 minutes per hour and 9.5 hours per day. Likewise, this corresponds to 292 hours per ICU bed and year. Due to lack of staff and/or excess workload, the manual recording of UO is usually not possible in all wards where it would have been needed. Thus, patients at risk of developing AKI may be neglected.

There are several risks associated with incorrect manual UO recording. First, registrations may not be carried out at exactly a full hour, leading to a false hourly UO measurement. For example, a 5 minute-error is introduced if one measurement is taken 3 minutes before full hour and the subsequent measurement is taken 2 minutes after full hour. This translates into an error of 8%. In one study of manual UO, the mean time error in routine ICU monitoring was found to be 16% ±15% (24). Second, the visual assessment of the urine level must be done meticulously keeping the eyes at the level of the urine, which otherwise leads to a false recording. Third, manual data-recording, whether by pencil or into an electronic patient data management system, is another source of error. In contrast to an automatic urinometer (AU), manual urinometers (MU) do not emit an alarm in case the system is mispositioned or recordings are not within the set reference range. Furthermore, manual emptying of the measuring chamber into the collection bag implies a theoretical risk of infection upon contact with the urinometer. The numerous obstacles involved in manual UO recording as depicted above, have questioned the trustworthiness of MU measurements (25).

## 1.3 AUTOMATIC URINOMETERS

Several AUs have been developed to facilitate hourly UO diuresis measurement, whereby different methods to measure UO have been applied. Measuring techniques used include droplet-based (23), electromagnetic switch (26), high precision scale (27, 28), air pressure based volumetric pump (Sentinel®, Serenno Medical, Israel), temperature exchange based electronic sensor (Clarity RMS™, RenalSense, Israel) (29), weight sensor (Sensica UO®, Adaptec Medical Devices, US), motor-pump, speed-based calculation (HS-UM1®, Hyupsung Medical, South Korea), artificial intelligence-driven system to support predictive analytics (Accuryn Monitoring System®, Potrero Medical, US) (24) and capacitance (30). Some AUs have not reached or seem to have disappeared from the market (23), and some

are being tested in clinical practice (Sentinel®, Serenno Medical, Israel). Most of these techniques show according to their manufacturer excellent performance, and high accuracy, including at low and high UO flow rates. However, very few techniques have been clinically validated and usually results of ongoing or planned studies have not been published in international medical journals. We have only found one technique in current clinical use that has been clinically validated (29), except for the capacitance technique, which is evaluated in this thesis.

### 1.3.1 The capacitance technique for AU measurements (Sippi®)

The capacitance technique is used in an AU developed by a Swedish startup company (Sippi®, Observe Medical, Gothenburg, Sweden). This device comprises two units: a base unit to which a disposable unit is attached (**Figure 2**).

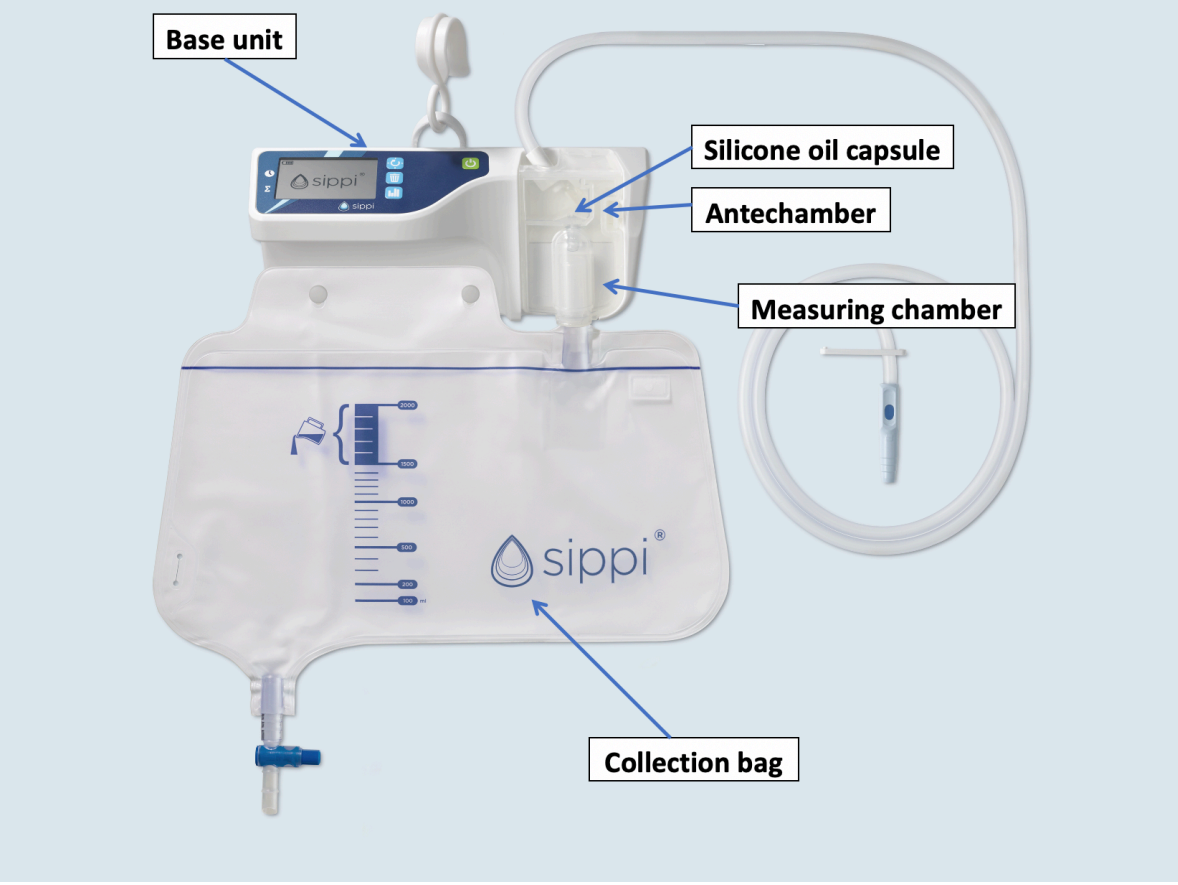



**FIGURE 2.** Overview of the evaluated new automatic urinometer (Sippi®, Observe Medical, Gothenburg). (Reprinted with permission from Observe Medical).

This AU is attached to the urinary catheter from where urine flows into the antechamber. In the antechamber, urine dissolves a capsule containing silicone oil that is transferred with the urine to the measuring chamber located just below. Change in capacitance between two

sensors is used to estimate the urine volume within the measuring chamber and the urine volume is constantly documented automatically. After measurement, when a volume of 16-18 mL is attained, urine will depart automatically from the chamber using a siphon technique to a collection bag.

### 1.3.2 Advantages with an automatic urinometer

An AU has several potential advantages compared with a traditional MU. First, an AU may reduce human error. Second, it should save the work load of the nursing staff (31, 32). Third, the applied electronic technique to measure UO may be used to warn for looming AKI in a complicated care environment (33) and also enables the implementation of kidney injury criteria such as AKIN and KDIGO. Fourth, the use of an AU should help clinicians to improve monitoring and forecasting the patient´s fluid balance. The importance of continuous monitoring of UO for AKI and fluid management in critically ill patients has been emphasized by leading nephrologists and critical care experts (12, 13, 17, 25, 34, 35). In a retrospective study of close to 16,000 ICU patients, intensive monitoring of UO (defined as hourly recordings and no gaps >3 hours for the first 48 hours after ICU admission) was linked to better detection of AKI and lessened 30-day mortality in AKI patients, in addition to a decreased fluid overload for all patients (23). Fifth, "no-contact" data transmission may lessen the risk of cross-infection of bacteria and virus, including SARS-CoV-2, between ICU patients and staff. Sixth, AUs may enable measurement of UO in normal wards. Commonly, staff shortage does not allow manual recordings of UO to a desirable degree and this may help to identify patients at risk of developing AKI. Seventh, minute-to-minute recording of UO may help to identify sepsis at an early stage (36).

### 1.3.3 Obstacles to introducing automatic urinometers

As discussed above, very few AUs are currently used clinically and only one has been clinically validated (29). The numerous techniques used for automatic urine measurements indicate the complexity in developing a solid measurement technique for clinical use. Apart from being accurate at various rates of urine flow, the AU should be easy to handle, robust, capable of measuring urine with varying concentrations in electrolytes, urea, creatinine, osmolality, glucose etc., as well as to be competitive in price and to allow communication with common patient data management systems.

The AU Sippi® is currently in use in several ICUs around Europe. However, every new device needs to be scientifically validated and our aim, in the first part of this thesis, was to validate this AU in both adult and pediatric patients.

## 1.4 BIOFILM

### 1.4.1 Definition

Biofilm is composed of extracellular polymeric substances (EPS) containing primarily polysaccharides, proteins and extracellular DNA (37). These are produced by most bacteria and candida (37, 38), enabling the microorganisms to be irrevocably accumulated and fixed to a surface, making their elimination difficult (**Figure 3**). Biofilm may be produced on both host tissue cells, such as uroepithelial cells, and abiotic surfaces, including urinometers and indwelling urinary catheters. As an example, in a study by Sabir et al., bacterial biofilm was found in 73.4% of patients having symptoms of a catheter-associated urinary tract infection, underscoring the impact of biofilm (39). If released from the biofilm, microorganisms may instigate life-threatening infections that need long hospital treatment.

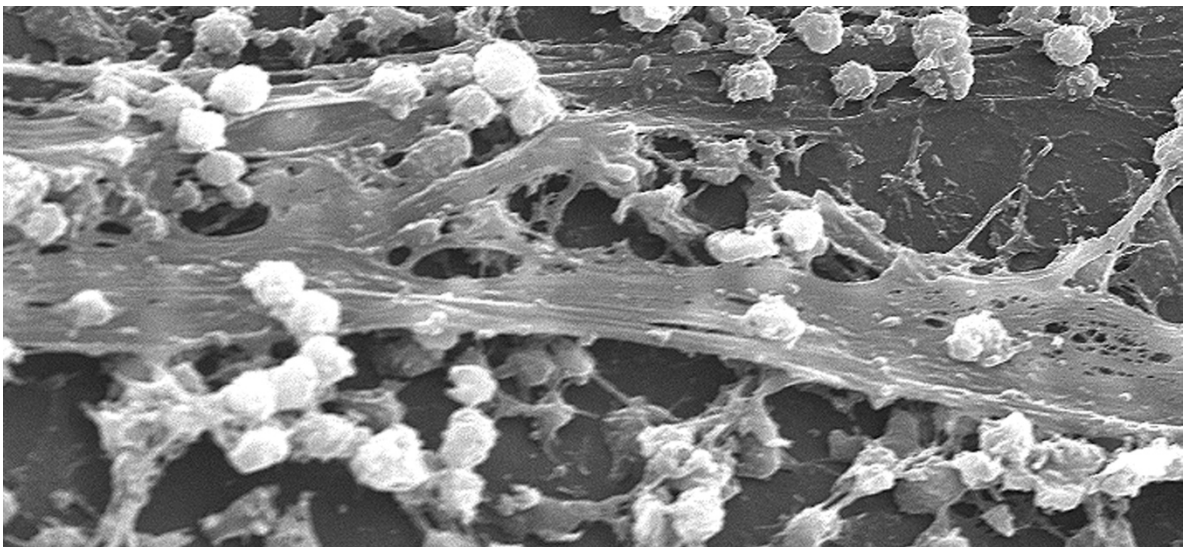


**FIGURE 3.** A scanning electron microscopic image showing Staphylococcus aureus bacteria on an indwelling catheter. Biofilm is seen as the sticky-looking substance between the round bacteria. (Reprinted with permission from Public Health Image Library/Centers for Disease Control and Prevention).

### 1.4.2 Biofilm formation

Biofilm is formed in several steps (40, 41) (**Figure 4**). In short, free-living (planktonic) microbes initially interact briefly and intermittently with a surface (reversible attachment). As microbes adapt to the surface, special surface-sensing features are expressed, the cyclic adenosine monophosphate level is increased within the cells and more and more cells will attach to the surface and remain so for longer periods of time (early biofilm formation). The cells will finally enter into an irreversible attachment stage, in which cells are stuck to the surface and start producing an extracellular matrix (mature biofilm). Finally, some microbes will be released from the biofilm (dispersal phase) and these microbes are often

phenotypically and sometimes also genotypically, transformed. Throughout this process, complex signaling is thought to take place between the microbes (42).

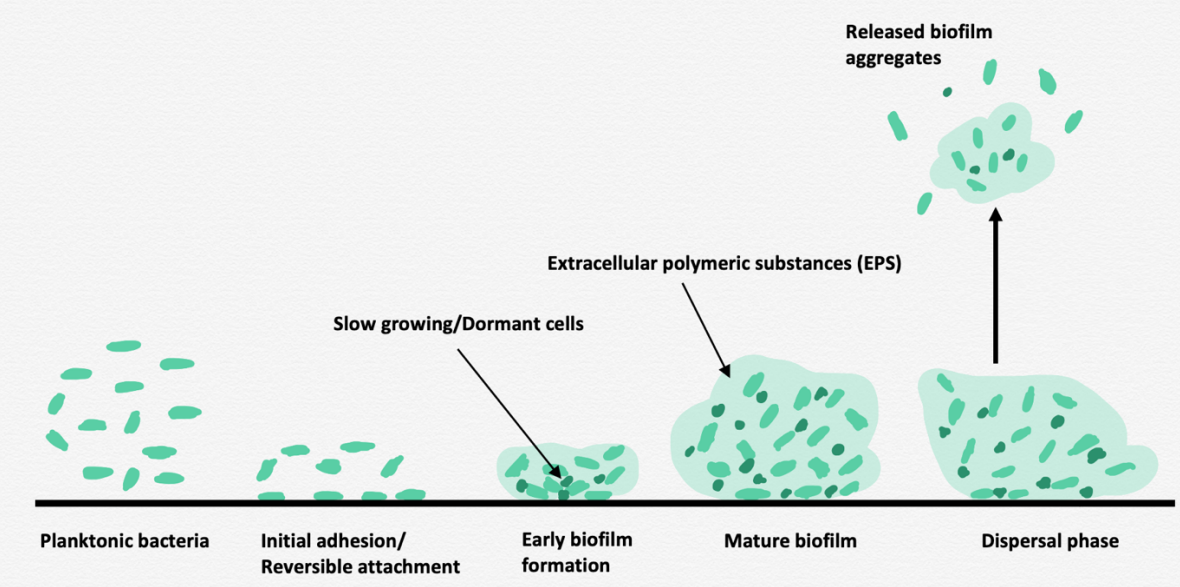



**FIGURE 4.** The different phases in biofilm formation. (Illustration by Martin Slettengren).

### 1.4.3 Virulence factors

Bacteria possess different virulence factors to resist the host defense mechanisms and to express biofilm. Those virulence factors, here exemplified by uropathogenic *Escherichia coli*, can be divided into different groups based on the underlying mode of action (**Figure 5**). Adhesins (e.g. fimbriae or pili) mediate adherence to host cells (43). Toxins (e.g. lipopolysaccharides and haemolysin) may destroy host immune effector cells and enable the bacteria to penetrate deeper into issue. Iron acquisition mechanisms (e.g. siderophores) are vital for the bacteria to survive in the iron-depleted environment of the urinary tract. Immune evasion (e.g. cellulose) is used to escape from the host immune cells by biofilm production, intracellular hiding or to suppress the pro-inflammatory response. Finally, *E. coli* is equipped with multiple rotating helical filaments, termed flagella, enabling the bacteria to swim, for example, against the direction of the urine flow (44).

In **Study III** we investigated the effect of different virulence factors of uropathogenic *E. coli* strains. More specifically, we studied type 1 fimbriae, curli and cellulose. Type 1 fimbriae, adhesins, are helical structures composed of several subunits. Those fimbriae are most commonly produced through the chaperone-usher pathway in which chaperones fold the different subunits of the fimbriae in the periplasm of the bacteria and subsequently a protein, the usher, assemble the fimbriae in the outer membrane (45). One such usher, which is essential to fimbriae biogenesis, is *FimD*.

Curli, an amyloid, is also an adhesin and a major part of the extracellular matrix, that promotes cell accumulation, adhesion to surfaces and biofilm formation (41). It is composed of a large number of the major subunit curli specific gene *(Csg)A*, which after polymerization is transported across the outer cell membrane to form the functional fiber exposed at the bacterial surface. This transport is mediated via the nucleator subunit *CsgB* (46).

Cellulose is a polymer consisting of repeating chains of glucose molecules linked via β-1,4 glycosidic linkages, synthesized by glycosyltransferases located in the membrane. Linked to the synthesis is membrane translocation through a channel constituted by cellulose synthase. This synthesis and translocation process are catalyzed in bacteria by the inner membrane-associated bacterial cellulose synthase *(Bcs)A* and *BcsB* subunits (47). Cellulose provides structure and protection to bacteria in biofilm through the formation of a matrix. Moreover, cellulose may help bacteria to reduce the host's immune response as well as cell aggregation, enabling the biofilm to reach the surface of the culture where oxygen is available to the bacteria (48).

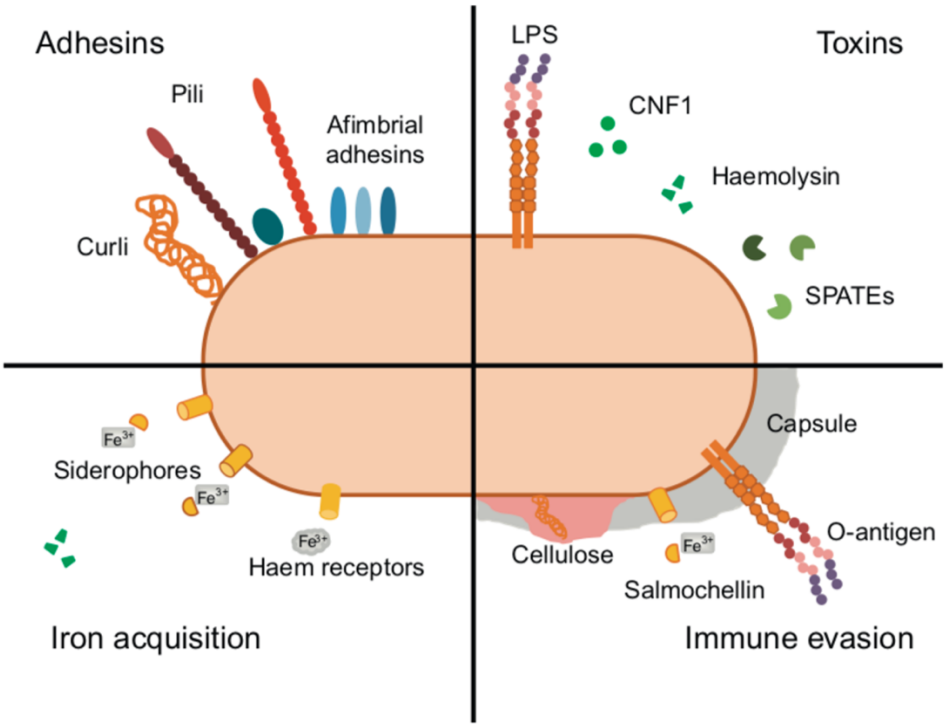



**FIGURE 5.** Virulence factors, including groups, of uropathogenic *Escherichia coli* (Reprinted with permission from Elsevier) (43).

### 1.4.4 Albuminuria

Normally, healthy kidneys only filter very small amounts of protein into the urine as almost all protein molecules are too large to pass through glomeruli. Proteinuria may be due to diseases of the glomeruli e.g. glomerulonephritis or diabetes mellitus, urinary tract infection, congestive heart failure, surgery or genetic differences in the glomerular endothelial function (49). Proteinuria is a known independent risk factor for AKI (50). In patients undergoing cardiac surgery, both preoperative (51) and postoperative (52) albuminuria can predict which patients have an increased risk of developing AKI during their hospital stay.

### 1.4.5 Hemoglobinuria

Hemolysis occurs due to destruction of red blood cells and results in release of hemoglobin into the blood system. The possible causes of hemolysis are many, e.g. toxins, hypersplenism, thrombotic thrombocytopenic purpura, autoimmune hemolytic anemia, bacterial infections, transfusion reactions and malignant hypertension. It may also occur during extended operations on CPB or extracorporeal membrane oxygenation (ECMO) as a result of mechanical forces e.g. shear stress, hypothermia, turbulent flow, excessive pump speed, cavitation or decreased oncotic pressure and clot formation, resulting in complete lysis or variable degree of damage of red blood cells (53-55). Excess free hemoglobin (fHb) in blood is filtered in the glomeruli of the kidneys that excrete it into the urine, which becomes dark red. Severe hemoglobinuria may result in acute tubular necrosis, acute renal failure and need for dialysis. In a study by Heijmans et al. (56), patients undergoing extended periods of CPB (valve + coronary surgery) had higher levels of plasma fHb than shorter periods (coronary surgery) during CPB and the first postoperative hours. In contrast, patients undergoing off-pump coronary surgery did not have increased levels of fHb in plasma. Patients with AKI (13.4%) exhibited significantly higher fHb serum levels already during surgery compared with patients without AKI.

### 1.4.6 Biofilm eludes antibiotics

Microbes form biofilm as an adaptation to external stressors such as the host immune response and antibiotics. Once biofilm is formed in the body, the acute microbial infection will progressively develop into a chronic infection that is difficult to treat and get rid of. Although antibiotics may work in early stages of biofilm, the effect is considerable weaker in mature biofilm and biofilm-growing microbes are 10 to 1000 times more resistant toward

almost all antiseptics and antibiotics (cell wall biosynthesis inhibitors), compared with planktonic microbes (57).

Several underlying mechanisms have been identified to cause this, e.g. slow or incomplete permeation of antibiotics through the biofilm to the bacteria, horizontal gene transfer between bacteria, creation of a new chemical microenvironment among the bacteria and the progress into a multicellular, heterogenous community of bacteria which is difficult for the antibiotics to reach and target (58). Also, microbes may enter into a tolerant state, making them capable to survive exposure to an excessive concentration of an antibiotic for some time. This may be due to the microbes entering a dormant state with minimal or no growth (59). It is estimated that around 80% of chronic and recurrent infections (58) and 65-80% of all clinical infections (60) are associated with biofilm.

### 1.4.7 Impact on medical devices

Microbial biofilm may deteriorate the function of medical devices, cause degradation of biomaterials and lead to nosocomial bloodstream infections (61, 62) with negative consequences for the patient. In the case of an implanted device with biofilm, such as a pacemaker or a mechanical heart valve, there is often no other option than to remove the infected device and reinstall a new device when the infection is under control (58). As for the AU using the capacitance technique, biofilm formation may result in false readings or even shutdown, stemming from the progressive biofilm coating of the measuring chamber that disturbs the capacitance signal (63). Plausibly, the biofilm may result in a higher risk of infection of the patient, regardless of the type of urinometer that is used. Preliminary clinical analyses of data in patients undergoing cardiac surgery using the AU Sippi® showed that measurements could not be recorded in some patients having albuminuria and/or hemoglobinuria or urinary tract infection after 24 hours use of the AU. Consequently, finding and validating new methods to decrease the formation of biofilm on medical devices, and in this case, the AU, is of clinical importance.

## 1.5 BIOFILM PREVENTION

### 1.5.1 Measures to decrease biofilm

Numerous substances and methods have been investigated with the aim to prevent, treat and control biofilm in clinical medicine. Methods range from surface modifications (64), antimicrobial impregnated surfaces (65), electricity (66, 67), and substances to prevent attachment of bacteria to a surface, such as cellobiose dehydrogenase/amylase (68), hydrogels (69), silver nanoparticles (70) and honey (71). Treatment may also target

established biofilm, e.g. signal interference between bacteria (72), antibiotic-loaded nanoparticles to enhance penetration into biofilm (73) and biofilm dispersion (e.g. Dispersin B) (74). Medical devices are also being developed that integrate a function of early biofilm detection (75).

### 1.5.2 Silicone oil

Silicones, also called polysiloxanes, are polymers consisting of repeating units of alternating silicon and oxygen atoms, linked to organic side-chains including carbon, hydrogen, and sometimes other elements. Silicone is used in a variety of applications, e.g. as lubricants, adhesives, cooking utensils, electrical insulation and in medicine. Silicone should not be confused with silicon, which is the chemical element with symbol Si and atomic number 14.

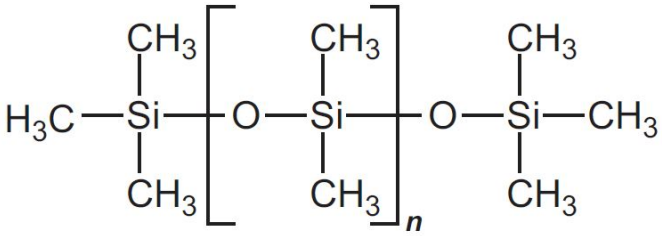


**FIGURE 6.** Principal chemical structure of the silicone oil polydimethylsiloxane (PDMS).


Silicones come in different forms, from hard plastic to liquid, e.g. silicone oil. Silicone oil is extensively used in medicine, for example in breast implants and in vitreous fluid substitutes. Safety studies indicate that silicone oil has a low toxicity and is associated with a low risk for adverse effects (76, 77). Moreover, silicone oil has no bactericidal effects on common microorganisms (78). The most prevalent type of silicone oil is polydimethylsiloxanes (PDMS) which is chemically inert, heat-resistant, non-toxic and has a low surface tension (**Figure 6**) (79). Interestingly, preliminary studies indicate that the silicone oil PDMS effectively reduce biofouling in polyurethane catheters and that long-standing slippery liquid infused porous surfaces can be produced on nano-surfaces that also inhibit bacterial formation (80, 81). As far as we know, the effect of PDMS has not been

investigated on polypropylene plastic, which is the plastic used in the measuring chamber of the evaluated new AU (Sippi®) and in parts of the MU (Unometer 500™) that are in contact with urine. From our clinical experience, by adding a water-soluble capsule containing silicone oil to the antechamber of the device, the problem we initially experienced with progressive biofilm formation and deteriorating capacitance signal of the AU Sippi®, seemed to be significantly postponed.

### 1.5.3 Polypropylene plastic

The siphon cassette of the studied AU, consisting of the antechamber and the measuring chamber, is made up of polypropylene plastic (PP), which is one of the most used plastics worldwide and largely utilized in medicine. It has the chemical formula (C3H6)n and is a thermoplastic polymer (**Figure 7**). PP is inherently hydrophobic and oleophilic (82). The tendency of biofilm formation varies between type of material. It seems like biofilm by some bacteria are more prone to form on hydrophilic surfaces, e.g. glass and stainless steel, than on hydrophobic surfaces, such as PP (83).

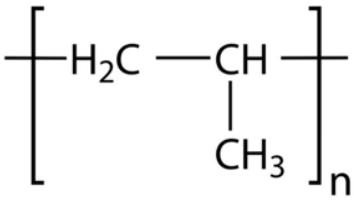



**FIGURE 7.** Principal chemical structure of polypropylene plastic (PP). (Reprinted with permission from Wikimedia Commons).

### 1.5.4 Biofilm prevention in the automatic urinometer Sippi®

Based on our initial clinical experience that an integrated water-soluble capsule containing silicone oil improved the capacitance measuring capability of the AU Sippi®, in particular for patients with urinary tract infections, albuminuria and hemoglobinuria, we sought to determine if the combination of PP and silicone oil had an effect in this regard. As a first step, we aimed to investigate this *in vitro*.

# 2 AIMS

The specific aims were to:

- Evaluate the performance of a new capacitance-based AU in adult patients in a cardiothoracic ICU and compare it with an MU in regard of bias, precision, temporal deviation and to evaluate the participating nursing staff's opinion of the AU compared with the MU.

- Compare a modified capacitance-based AU with an MU regarding bias of measurements, and to evaluate the participating nursing staff's opinion of the AU, among patients ≤10 kg in a pediatric intensive care unit.

- Investigate whether a silicone oil-coated polypropylene plastic surface, as used in an AU, may reduce early biofilm formation by pathogenic bacteria, including ESBL-producing and multidrug resistant strains, as well as *C. albicans*, and to investigate whether the viscosity of the silicone oil has an impact in this regard.

- Identify the tentative silicone oil target, by using an *E. coli* strain equipped with curli, cellulose and type 1 fimbriae and the isogenic mutants, deficient in one or more of these virulence factors.

- Investigate whether albumin or free hemoglobin coating of the capacitance measurement membrane of the AU could influence the capacitance measuring capability of the AU and whether this could be attenuated by adding silicone oil to the measuring chamber of the device.

# 3 METHODS

## 3.1 THE NEW AUTOMATIC URINOMETER

The new AU estimates the UO by measuring the change in height of a column of urine in a measuring chamber (**Figure 8A**), after the urine has passed through an antechamber (**Figure 8C**), which contains a water-soluble capsule with silicone oil (**Figure 8B**) of medium viscosity (viscosity 350 $mm^2/s$) (Silbione®, oils 70047, V350, Elkem, Oslo, Norway). Thus, the first urine from the patients will dissolve the capsule and transport the oil to the measuring chamber, which will get coated with a film of silicone oil. A capacitance-based sensor continuously records the height of the urine column through the polypropylene plastic wall of the measuring chamber. When the measuring chamber gets filled, the urine empties automatically via a siphon. The AU runs on 3 AA batteries, which need to be exchanged every three to four months. The standard display shows the accumulated UO during the present hour, UO from the last hour and the accumulated UO during the present 24-hour period (**Figure 9**). By pressing a button, each hourly UO of the present 24-hour period can be presented as a graph with columns. Optionally, stored data can continuously be transferred via Bluetooth to a patient data management system (https://observemedical.com/sippi/).


**FIGURE 8.** The measuring chamber (A, lower 2/3) of the automatic urinometer (Sippi®, Observe Medical, Gothenburg, Sweden) with a water-soluble capsule (B) containing medium viscosity silicone oil in its ante-chamber (C) upper 1/3). (Reprinted with permission from Observe Medical).


**FIGURE 9.** The display of the evaluated automatic urinometer (Sippi®, Observe Medical, Gothenburg, Sweden). (Reprinted with permission from Observe Medical).

### 3.1.1 Capacitance measurement

Capacitance, by definition, is the ability of a component to store an electrical charge and is measured in the SI unit farad (symbol: F). Such a component is called a capacitor and consists of two metal plates with an insulating layer in between, called dielectric. If a 1 F capacitor is charged with 1 coulomb (1 ampere x 1 second) of electrical charge, it will have a potential difference of 1 volt between its plates. The capacitance of a capacitor is determined by the size and the distance between its plates and the amount and type of dielectric. In the AU Sippi®, the dielectric consists of a column of urine in the measuring chamber and the higher the height of urine column in the measuring chamber, the higher the capacitance. By measuring the duration it takes to charge and discharge the capacitor, a value is acquired of the capacitance that is related to the height of the urine column in the measuring chamber. The volume of urine in the measuring chamber can easily be calculated based on the change in capacitance. The measurement resolution is 1 mL. In our studies we read the visual information on the display every hour according to the inbuilt clock. Thus, we could record the information about hourly diuresis at any time. The hourly diuresis cycle was determined by the internal clock. The automatic urinometer data can be automatically transferred to a patient data management system via Bluetooth, as required, usually every solar hour.

Two capacitance sensors are used, one main sensor (Cm) and one reference sensor (Cr). If there is a linear urine inflow to the measuring chamber, Cm will increase, followed by an augmentation of Cr in the middle (**Figure 10**, between A and B), and then Cm will rise

again. Due to the automatic emptying of the measuring chamber by the built-in siphon, the signals from Cm and Cr will decrease rapidly when the chamber self-empties. Nevertheless, several external factors, e.g. tilting of the system or biofilm formation within the measuring chamber, will affect the signal. The capacitance changes are very low, close to $10^{-12}$ F.

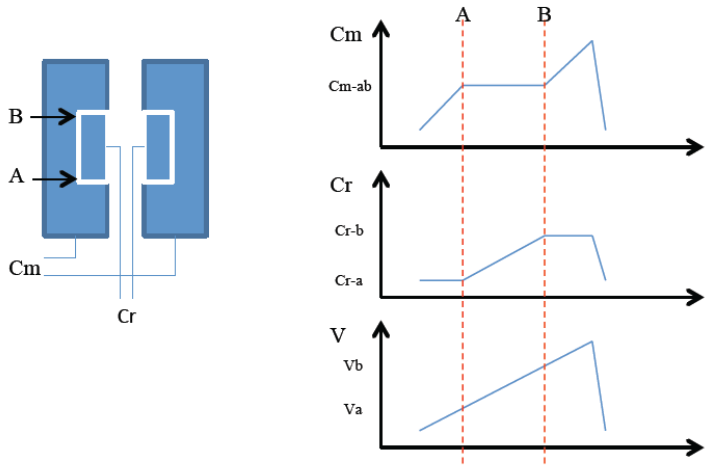



**FIGURE 10.** The rise in capacitance over time when the urine volume increases in the measuring chamber. Cm = Main plate. Cr = Reference plate. (Reprinted with permission from Observe Medical).

### 3.1.2 Siphon effect

A siphon is a liquid reservoir equipped with an inverted U-tube. First, the inverted U-tube is filled with liquid after which the siphon may drain liquid from the reservoir to a higher level than the reservoir surface and then down again to a lower level. It will do so continually without external energy until the level in the reservoir falls below the level in the output end of the U-tube (**Figure 11**).

The mechanism of the siphon effect has been debated (84, 85). In the past, the atmospheric pressure was attributed to push water through the tube, whereas a more recent explanation is based on gravitation: The column of water in the downward tube drags water up in the upward tube and acts like a chain with the water molecules interacting with each other using hydrogen bonds. The maximum height of a liquid siphon depends on the tensile strength of water – i.e. the maximum weight that hydrogen bonds are able to lift. It is critical that the outflow of the tube lies lower than the inflow of the tube, in that case gravitational energy is released at the bottom of the tube and that energy will drag the water in the upward tube. If air comes into the tube, the siphon effect will be broken. The siphon effect is used in common water closets.

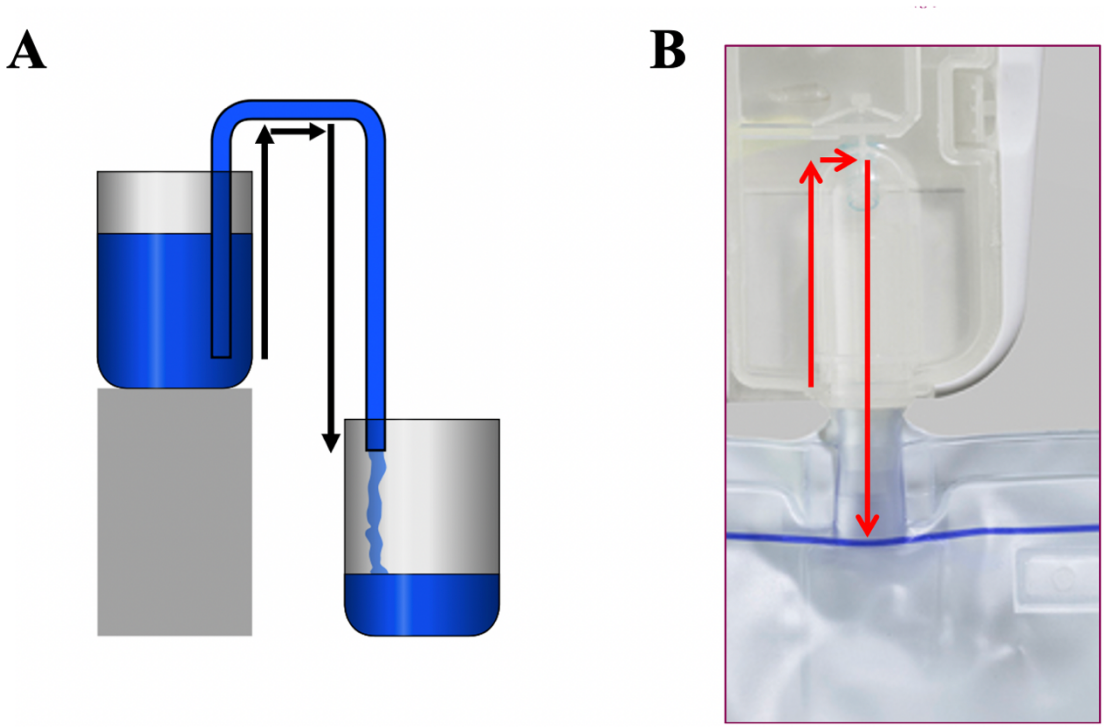



**FIGURE 11.** The principle of the siphon effect (A) and how it is constructed in the automatic urinometer (B). (A. Reprinted with permission: User: Tomia, CC BY-SA 3.0 <http://creativecommons.org/licenses/by-sa/3.0/>, via Wikimedia Commons; B. Reprinted with permission from Observe Medical).

The AU Sippi® uses the siphon effect to transport urine from the measuring chamber to the urine collection bag. The measuring chamber empties at a volume around 16-18 mL, and the exact volume will be registered each time.

### 3.1.3 Signal processing algorithm

An algorithm is used to calculate the urine volume from the measured capacitance values. By using signals from the two sensors, the algorithm self-calibrates the device after each UO measurement. For every new measurement the AU will sense the existing conditions and adapt accordingly.

### 3.1.4 Biofilm warning

Biofilm that is accumulating on the plastic wall of the measuring chamber close to the capacitance measurement membrane will affect the capacitance measurement and increase the capacitance signal. Biofilm will form from the bottom to the top, resulting in progressive failure of the sensors in the lower part of the measuring chamber. This triggers an alarm when reaching critical levels that creates a risk of misreading (**Figure 12**).

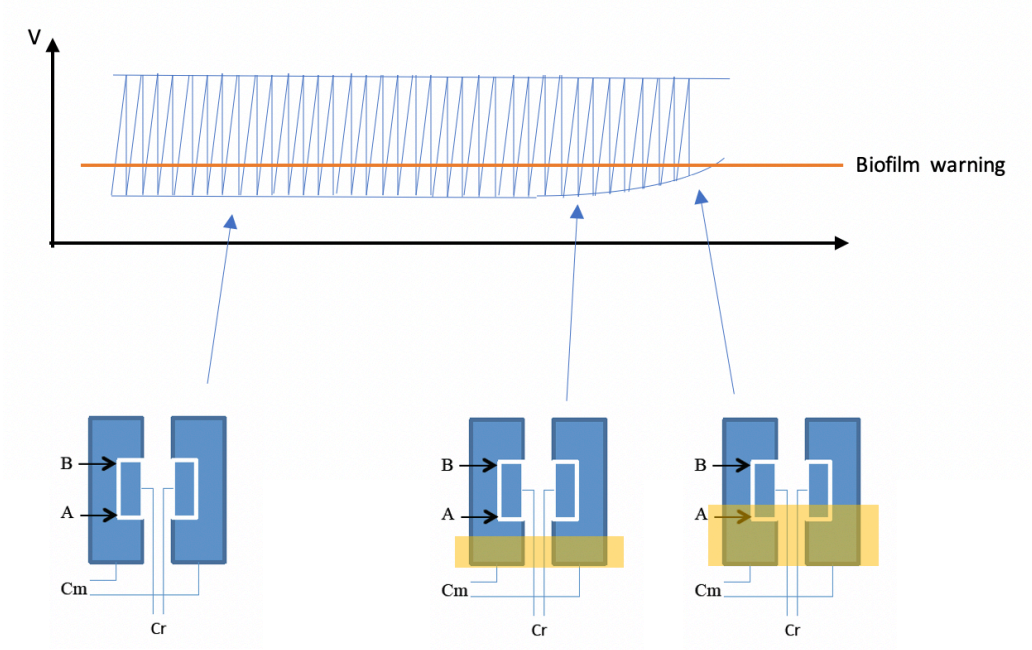



**FIGURE 12.** Progressive biofilm build-up (yellow areas) begins at the bottom of the measuring chamber and progresses towards the top. The capacitance expressed as voltage (V) undulates each time the measuring chamber is filled and emptied, from A to B. If biofilm is formed, it will occur from the bottom to the top of the measuring chamber, resulting in progressive failure of the sensors in the lower part of the measuring chamber. This triggers an alarm when reaching critical levels that creates a risk of misreading Cm = Main capacitance sensor. Cr = Reference capacitance sensor. (Reprinted with permission from Observe Medical).

## 3.2 CLINICAL STUDIES

### 3.2.1 Study design

**Study I** and **Study II** were prospective observational cohort studies performed in the cardiothoracic ICU at Karolinska University Hospital, and in the pediatric intensive care unit at Astrid Lindgren's Children Hospital, Stockholm Sweden, respectively. **Study I** included 34 adult patients who had undergone cardiac surgery, whereas **Study II** comprised 12 patients, with an indwelling urinary catheter before inclusion, weighing ≤10 kg, which corresponds to ≤12 months of age (86). Based on **Study I**, we aimed to have about 100 cylinder and urinometer measurements for each device, the MU and the AU, in **Study II**. Apart from evident exclusion criteria such as anuria and on-going dialysis, we did not have any exclusion criteria for **Study I** and **Study II**.

### 3.2.2 Hourly urine measurements with the AU, MU and reference cylinder

A laboratory technician measured the reference urine volume with a laboratory precision measuring cylinder immediately after each hourly measurement with the AU or the MU. This person was unaware of the data from the urinometer measurement till after the corresponding reference measurement, but aware of which urinometer that was used. Immediately prior to each full hour, the AU tube and the MU tube, respectively, were emptied. Next, the technician momentarily blocked the tube close to the measuring chamber inlet in order to prevent urine inflow during data recording and urine gathering. Thereafter, the attending nurse noted the data from the AU screen. Afterwards, the technician emptied the attached urine plastic pouch and evaluated the urine volume with the reference cylinder. The urine volume that remained in the measuring chamber of the AU, when reference measurements had been made, usually varied somewhat between two successive measurements. This influenced the measurement of the reference volume. For this reason, the technician used a transparent plastic measuring scale delivered by Observe Medical (accuracy ±1 mL) to register the remaining urine volume in the measuring chamber at each measurement. The temporal deviation of the MU was investigated during a separate period in the ICU, by registering the exact time of each measurement (**Study I**). We paired data from each urinometer and from control cylinder used as a reference.

The MU stores urine in graded chambers, which enables visual reading of the accumulated urine volume by the attending nurse before the chambers are emptied manually. A prerequisite for correct measurements is that the MU hangs vertically and that the grading scale lines are in a horizontal position. In contrast, the AU senses changes in positions that interfere with measurements, via a built in accelerometer, and displays an error message on its display. Also, an error message appears on the display of the AU when the disposable unit needs to be replaced, usually after 7 days or earlier due to severe biofilm formation in the measuring chamber.

### 3.2.3 Measurements in adult patients (Study I)

An indwelling urinary catheter was inserted on all adult patients participating in **Study I** in the operating room after induction of anesthesia according to clinical practice. After arrival to the ICU, we connected the patients to either the AU (Sippi®, Observe Medical, Gothenburg, Sweden) (**Figure 2, 8, 9**) or to a standard MU (UnoMeter™ 500, Unomedical a/s, Birkeroed, Denmark, **Figure 13A**).

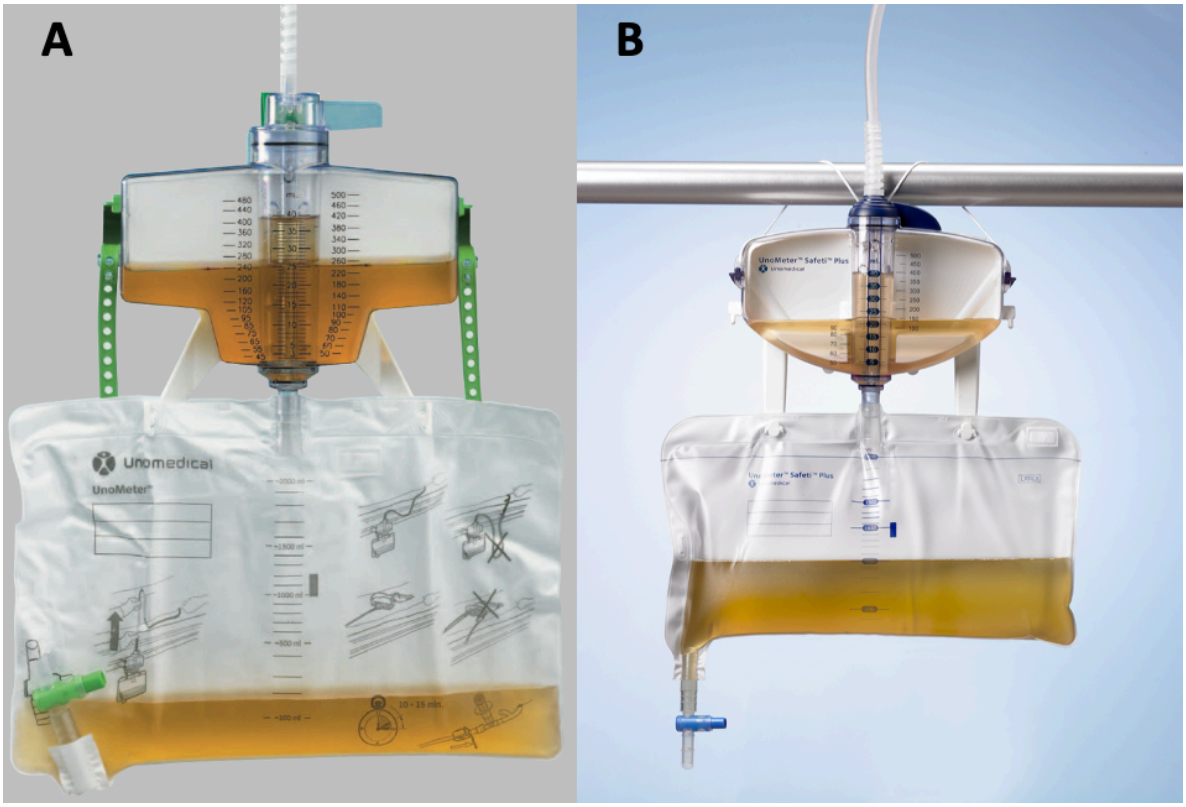



**FIGURE 13. A.** The manual urinometer UnoMeter™ 500 (**A, Study I**) and the manual urinometer Unometer™ Safeti™ Plus (**B, Study II**). (Reprinted with permission from Convatec).

We used a laboratory measuring cylinder (250 mL; BLAUBRAND®, BRAND GMBH + CO KG, Wertheim, Germany) with a tolerance of ±1 mL as a control. We evaluated the urinometers during two separate time periods, first the MU to reflect routine UO measurements followed by the AU. There were no cross-overs between the studied urinometers. We registered UO data from each patient every hour during daytime for up to 3 consecutive days, without demanding any minimum number of measurements to be entered in the analysis.

### 3.2.4 Measurements in pediatric patients (Study II)

We measured UO in the studied pediatric patients with an AU (Sippi®, Observe Medical Nordic AB, Gothenburg, Sweden) and an MU (Unometer™ Safeti™ Plus, Convatec Inc., Lejre, Denmark) (**Figure 13B**) and analyzed and compared data with a gold standard measuring cylinder (25 mL; Pyrex® DIN 12680 25:0.5, England).

The included 12 children either received an AU or an MU, although we registered UO in 2 children with both urinometers. We measured UO every hour during daytime for up to 4 sequential days. Clinical data are presented in **Table 7**.

To make the AU and the MU in **Study II** more comparable, we substituted the original single lumen tube of the AU, connected to the indwelling catheter, with a sterile double-lumen tube. This is the same kind of tube applied in the MU, as it is devised for measuring small urine volumes in pediatric patients (**Figure 14**).

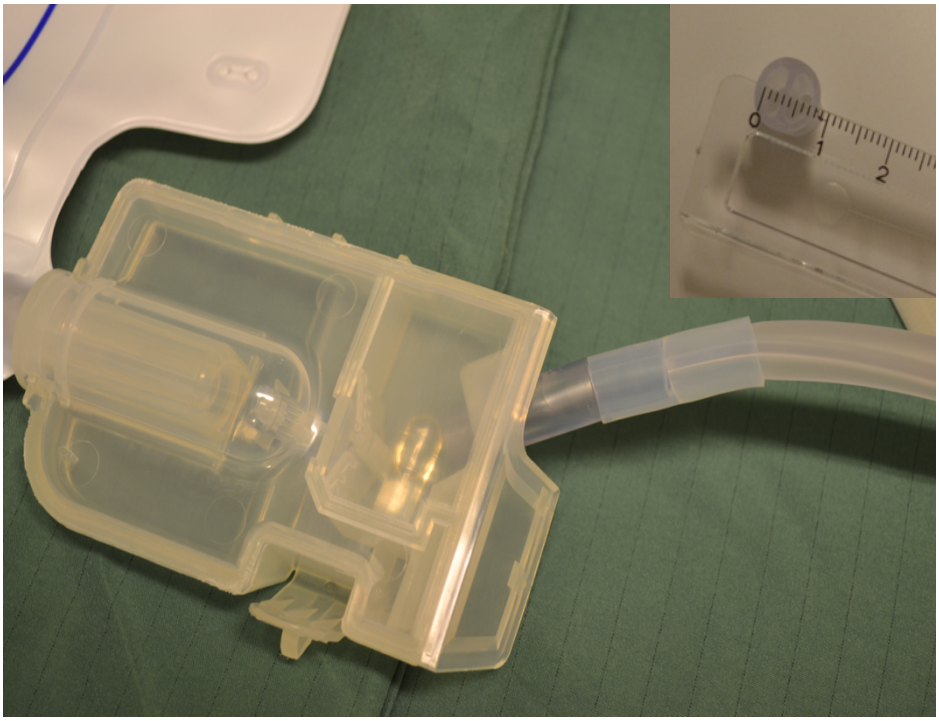


**FIGURE 14.** The double-lumen tube between the patient´s indwelling urinary catheter and the automatic urinometer in **Study II**. (Photo by Martin Slettengren).


**FIGURE 15.** The automatic urinometer in clinical use, mounted on a pediatric patient's bed.
(Photo by Martin Slettengren).

### 3.2.5 Staff view of the urinometers (Study I and II)

We investigated the staff's view of the urinometers after ending **Study I** and **II**. Each participating nursing staff member answered an anonymous questionnaire (**Table 6** and **Table 9** in **Results**) after a 15-minute introduction course and having used the AU during 3 days in the respective ICU. The questionnaire included 5 questions considering the easiness to learn how to use the AU (question 1) and the user easiness of the AU compared with the MU (questions 2-5). There were no missing data. For each question, an ordinal scale ranging from 1 to 5 was used. Answers to question 1 were analyzed separately, whereas questions 2 to 5 were analyzed by aggregated and personal mean.

## 3.3 EXPERIMENTAL STUDIES

### 3.3.1 Study design

**Study III** and **IV** were experimental prospective in-vitro studies.

### 3.3.2 Bacterial and fungal biofilm formation (Study III)

*3.3.2.1 Bacteria and candida strains*

In **Study III** we included the uropathogenic Gram-negative bacteria *E. coli* #12, from a child with acute pyelonephritis, *E. coli* strain CFT073, from a patient with acute pyelonephritis, extended spectrum beta lactamase (ESBL)-producing *E. coli* (CCUG 55971), *Proteus mirabilis* (ATCC 29245), *Klebsiella pneumoniae* (ATCC 13883) and multi-drug-resistant (MDR) *Klebsiella pneumoniae* (CCUG 58547), *Pseudomonas aeruginosa* (ATCC 27853) and the Gram-positive bacteria *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 29213) and finally the fungus *Candida albicans* (CAC4). *E. coli* #12, wild-type strain, is equipped with curli, cellulose and type 1 fimbriae, which are essential for production of biofilm. To investigate if silicone oil targeted any of these virulence factors, we utilized isogenic mutants, lacking at least one of the virulence genes. The set of *E. coli* strains included the wildtype strain #12 (curli+/cellulose +/type 1 fimbriae+) and its isogenic mutants WE1*bcsA* (curli+/cellulose−/type 1 fimbriae+), WE11*csgBA* (curli-/cellulose+/type 1 fimbriae+) and WE16*csgBA* bscA (curli−/cellulose−/ type 1 fimbriae+) (87, 88). Type 1 fimbriae were verified by yeast agglutination. We created a *fim D* deficient strain (WK*fimD*) by the λ-Red mediated homologous recombination method (88, 89) to validate the precise outcome of the silicone oil on type 1 fimbriae.

*3.3.2.2 Preparation of bacteria and C. albicans*

In **Study III** we cultured *E. coli* #12 and its isogenic mutants on Luria-Bertani (LB) agar plates without salt for at least 24 hours to stimulate biofilm formation, while we cultured other bacterial strains overnight at 37ºC on blood agar plates. Single bacterial colonies were applied for bacterial suspension preparation in phosphate-buffered saline. To circumvent bacterial aggregates, we centrifuged the suspension at 1000 revolutions per minute (RPM) for 5 min and measured the suspension's optical density at 600 nm using a spectrophotometer and adjusted the suspension to a final concentration of $10^6$ colony forming units (CFU) per mL in LB broth without salt. The bacterial concentration was verified by viable count. We cultured *C. albicans* in YPD (yeast peptone dextrose) using the same protocol as for bacteria.

*3.3.2.3 Biofilm formation on polypropylene and polystyrene plastic*

To compare the amount of biofilm formed on polypropylene plastic without silicone oil with another frequently used plastic, polystyrene, we added *E. coli* #12 to wells in these plastics and left them for 72 hours, where after we measured biofilm formation with a crystal violet assay (see below). We studied the effect on polypropylene plastic because the measuring chamber of the AU (Sippi®, Observe Medical, Gothenburg, Sweden) consists of this plastic.

*3.3.2.4 Silicone oil*

In **Study III** we used a low-viscosity silicone oil (viscosity 100 $mm^2/s$) and a medium-viscosity silicone oil (viscosity 350 $mm^2/s$) (Silbione, oils 70047, V100 and V350, Elkem, Oslo, Norway). The latter oil was also used in **Study IV**. The medium-viscosity silicone oil is used within the measuring chamber of the AU Sippi®, evaluated in **Study I**, **II** and **IV**.

*3.3.2.5 Pretreatment of microtiter plate with silicone oil*

In **Study III** we pretreated 96 well clear flat-bottom polypropylene microtiter plates (Sigma, USA) with either 300 µl of low viscosity silicone oil (V100) or medium viscosity silicone oil (V350) in each well for 5 minutes. Thereafter, we immediately and meticulously removed as much oil as possible from each well with a disposable micro-pipette, leaving merely a thin coating of silicone oil in each well.

*3.3.2.6 Measurement of biofilm formation with crystal violet*

The preparation of bacterial suspension has been described earlier (87). In short, we added 50 µl of $10^6$ CFU/ mL of bacterial suspension in LB broth without salt together with 150 µl

of LB broth without salt, giving a total volume of 200 µl in each polypropylene well with and without pre-treatment with the low and medium viscosity silicone oils. We then incubated the microtiter plates at 37˚C without shaking for 72 hours. After incubation, we removed planktonic cells (single free moving or swimming cells in the medium), washed the plates twice with PBS and let them dry in air. We then studied the effect of silicone oil on biofilm formation using the crystal violet assay (90). Each well was stained with 220 µl of 0.3% crystal violet for 5 minutes, and then destained them with 250 µl of 20% acetone and 80% ethanol. The optical density of dissolved crystal violet was determined at 570 nm. In **Study III** all bacterial strains and *C. albicans* were studied with the same protocol. We compared silicone oil treated wells with the untreated controls. To evaluate the viability within the biofilm, selected microorganisms, *E. coli* #12, *P. aeruginosa*, *S. aureus* and *C. albicans,* were allowed to grow and form biofilm for 72 hours.

#### *3.3.2.7 Exclusion of a direct bactericidal or fungicidal effect of silicone oil*

In **Study III**, we performed growth curves of *E. coli* #12, *S. aureus* and *C. albicans* in order to exclude a direct bactericidal or fungicidal effect of the medium viscosity silicone oil. 50 mL polypropylene Falcon tubes were coated with medium viscosity silicone oil for 5 minutes, followed by incubation of *E. coli* #12 or *S. aureus* in LB broth at 37˚C for 15 hours, *C. albicans* in YPD broth at 30˚C for 24 hours. We performed viable counts after 3, 6, 9 and 15 hours for *E. coli* #12 and *S. aureus,* and at 3, 6, 9, 12 and 24 hours for *C. albicans* post incubation with and without oil after serial dilution on blood agar.

#### *3.3.2.8 Effect of silicone oil on C. albicans hyphae*

*C. albicans* staining was performed from overnight grown cultures in YPD broth cultured with and without oil at 30ºC and centrifuged at 100 RPM. A thin smear was formed on the glass slide using an inoculation loop. We let the slides dry for 30 min at 55ºC. We then added one drop of blankophor p to the smear and the slides were instantly evaluated using ultraviolet light with an Olympus microscope with a 20X objective.

### 3.3.3 Albumin and free hemoglobin biofilm (Study IV)

#### *3.3.3.1 Experimental setup*

In **Study IV** we explored the effect of silicone oil on biofilm production of albumin and fHb, each diluted in a separate solution. The studied solution was stored in a 2500 mL container with an outgoing tube, which passed through a peristaltic pump (WELCO WPX1-P3/328; WELCO Co., Ltd, Tokyo, Japan) powered by a SPS 8041 (Manson Engineering Industrials Ltd, Hong Kong, China) with a default of 4.5 Volts. The outgoing tube was then

connected to the ingoing tube of the AU (Sippi®) device. To prevent vacuum, air was let into the container through a small hole, created with a 18G needle. The solution was thus run through the antechamber of the AU and then to its disposable collection bag (**Figure 16**).

The circuit was assembled with either the standard silicone oil capsule in the antechamber of the AU or not. The silicone oil capsule was dissolved when the studied solution entered the antechamber, whereby the silicone oil was transferred by the solution to the measuring chamber, where it adhered to its polypropylene plastic walls (91). Alternatively, the silicone oil capsule was removed through a hole created by a soldering iron in the front of the antechamber. Thereafter, the hole was sealed with a 3M tape designed for plastic surfaces. Every 24 hours a new solution was added to the container and the collection bag was emptied.

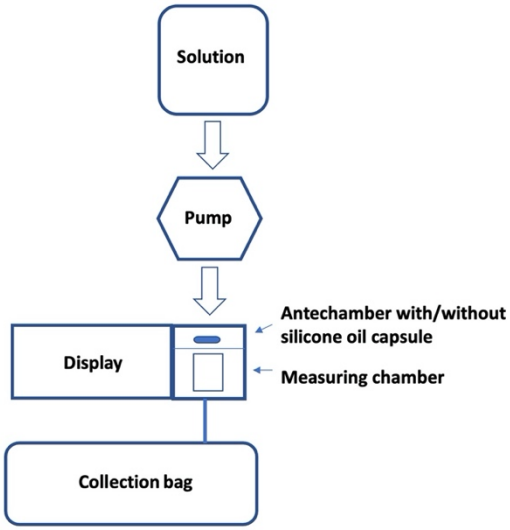



**FIGURE 16.** The experimental setup in **Study IV**. (Illustration by Martin Slettengren).

### *3.3.3.2 Albumin solution*

The first part of **Study IV** investigated the effect of an albumin solution on capacitance measurements with and without silicone oil released from a capsule. Sixty mL of albumin (Alburex® 50 g/L, CSL Behring AB, Danderyd, Sweden) was diluted in 960 mL Ringer's Acetate (Baxter International Inc, Deerfield, U.S.), giving a concentration of 3 g albumin/L. A new mixture with the same concentration, for each Sippi® peristaltic pump system, was produced for every 24 h measurement. The mixture was stored in a 2500 mL container as

described above. The peristaltic pump was calibrated to achieve a flow rate of approximately 42 mL/h, resulting in an estimated total protein concentration of 3 g/24 h. The albumin-Ringer's Acetate solution was conducted with two parallel groups, 20 times with silicone oil and 20 times without. Moreover, two additional experiments with a lower albumin concentration, 0.3 g/L and 1.0 g/L, respectively, were conducted.

#### *3.3.3.3 Free hemoglobin solution*

The second part of **Study IV** investigated the effect of fHb on capacitance measurement with and without silicone oil released from a capsule. fHb was acquired from blood remaining in syringes after routine arterial blood gas analysis in patients. Thirty-nine separate syringes were used. The residual blood from the syringes was centrifuged with a Sigma 1A (Axel Johnson Instruments AB, Stockholm, Sweden) at 3200 RPM for 10 minutes. The bottom layer, consisting of erythrocytes, was then extracted with a RAININ Pipet-lite SL1000 dropper (Rainin Instruments LLC, Oakland, U.S). In order to achieve lysis through osmosis, the erythrocyte concentrate was mixed with sterile water giving a volume of 10 mL. The fHb concentration was then measured with HEMOCUE® PLASMA/LOW Hb 201+ Hemoglobin spectrophotometer (HemoCue America, Brea, U.S.). The 10 mL fHb mixture was added to 990 mL of Ringer's Acetate and stored in 2500 mL containers as described above. Every 24 h, a new mixture, for each Sippi® peristaltic pump system, was produced and used in the same way as before with continuous capacitance measurements for 24 h. The fHb solution experiments were conducted 20 times with silicone oil and 20 times without.

#### *3.3.3.4 Extraction of data from the AU*

Measurements of capacitance in the measuring chamber were conducted 60 times/h, i.e. 1440 times/24h, and data were stored on a removable micro-SD memory card inside the device. Analysis of the data was carried out after each 24 h run. In order to reach the micro-SD memory card, the batteries of the Sippi® base unit were removed with a SANDVIK 7890 nippers (SNA Europe, Enköping, Sweden). The card was then inserted into a micro-SD card reader and data transferred to an Excel file. Every 24h, a new disposable set was used, except for the 2500 mL container, which was reused after cleaning in a GETINGE 600 series industrial washer (Getinge Group, Gothenburg, Sweden). Before new tubes and disposable sets were connected, the hardware unit was reset and the WELCO pumps and the SPS 8041 power unit were calibrated so that all used pumps operated at the same speed at 4.5 volt. The containers were refilled with new solutions and the pumps were restarted.

Careful initial monitoring of the circuit ensured that it worked as expected and that fluid dissolved the silicone oil capsule correctly.

#### *3.3.3.5 Analysis of capacitance data*

The Sippi® registers capacitance twice every second. Capacitance is the ability to store electrical charge and is affected by the height of urine in the measuring chamber, which can then be converted by the device to a volume. A mean of the two measurements is logged to the micro-SD card of the device once every minute. From these raw data (1440 measurements/day) the lowest value from every 60-minute period was extracted and stored in an Excel file, resulting in 24 measurements from every pump system a day. Twenty runs with two parallel systems (one with and one without a silicone oil capsule) yielded a total of 480 measurements for each group (24x20). Each stored capacitance value was the lowest starting point of every hourly capacitance measurement and represented, compared with the initial starting point, the increase in capacitance due to biofilm coating of the inner surface of the measuring chamber by albumin or fHb. When the baseline value went up, it indicated the growth of biofilm coating. Eventually it reached a critical point after which measurement of urine was no longer possible.

## 3.4 ETHICS

The research papers in the thesis followed the principles of the Helsinki Declaration. The Regional Ethical Review Board in Stockholm endorsed the studies (**Study I:** 2012/31-31/2; **Study II**: 2015/666-32; **Study IV**: 2015/666-32, 2015/2351-32). In **Study I**, patients were included after giving written informed consent, whereas in **Study II**, involving 12 children, informed consent was obtained from at least one parent before inclusion. **Study III** was a pure *in vitro* study on bacteria without need of ethical approval.

## 3.5 STATISTICS

Data were analyzed with SPSS® statistical program (IBM Corporation, Armonk, NY, USA) (**Study I, II** and **IV)** and with GraphPad Prism version 5.02 (GraphPad Software, San Diego, CA, USA) (**Study III)**.

**Study I** and **II.** We compared variables of patient groups with Student's t-test when normally distributed and the Mann-Whitney U-test when not and Fisher's exact test if data were binary. We used Bland-Altman plots to calculate agreement of the AU and the MU, respectively, with cylinder measurements (92-94). Data points show the difference between paired measurements. The mean deviation between the respective urinometer and the

cylinder formed the mean bias. We drew horizontal lines at mean bias as well as the upper and lower limits of agreement (LOA). The LOAs consisted of mean bias ±1.96 x standard deviation (SD). The mean bias and the SD are equivalent to the agreement of the AU and the MU. We applied an independent samples t-test to test for equality of mean bias, and Levene's test to test for equality of variances in the sample. We estimated absolute value of the deviation from exactly one hour between measurements and analyzed by mean, SD and 95%-limits of agreement. We used the one-sample t-test to test for significance of the staff evaluations.

**Study III.** Statistical analysis was performed with One-way ANOVA and Bonferroni post-hoc test to compare multiple groups. One sided difference with $p<0.05$ was considered significant.

**Study IV.** Mean and SD were used for descriptive purposes. Group differences were assessed with the Mann-Whitney U-test (unpaired) when non-normal distribution in continuous variables was ascertained. A p-value of $<0.05$ was considered significant. All p-values were two-sided.

# 4 RESULTS

## 4.1 STUDY I

We included in total 408 hourly UO measurements, 220 with the AU and 188 with the MU, respectively, from 34 patients, 18 in the AU group and 16 in the MU group (**Table 3**).

**TABLE 3.** Clinical variables of the patients in the automatic urinometer (AU) group and the manual urinometer (MU) group.

| Variable | | AU (n=18) | | MU (n=16) | | p |
|---|---|---|---|---|---|---|
| Female | % | 28 | | 50 | | 0.29 |
| Age | (years) | 68.0 | (64.3-75.5) | 66.5 | (63.5-73.5) | 0.75 |
| Weight | (kg) | 79.2 | ±15.3 | 80.3 | ±7.9 | 0.83 |
| Height | (cm) | 174 | ±5.8 | 172 | ±9.8 | 0.48 |
| BMI | (kg/m²) | 1.9 | ±0.2 | 2.0 | ±0.2 | 0.74 |
| Euroscore II | (%) | 1.7 | (0.9-2.6) | 1.6 | (0.9-3.1) | 0.85 |
| Preoperative albumin | (g/L) | 39.0 | (36.0-40.3) | 38.0 | (36.0-39.8) | 0.60 |
| Preoperative creatinine | (µmol/L) | 82.5 | (67.8-97.8) | 86.5 | (62.5-100) | 0.83 |
| Preoperative eGFR | (mL/min) | 78.0 | (62.4-104) | 75.4 | (67.8-89.4) | 0.91 |
| IDDM | % | 6 | | 6 | | 1.00 |
| COPD | % | 6 | | 6 | | 1.00 |
| LVEF <50% | % | 33 | | 31 | | 1.00 |
| Procedure | | | | | | |
| CABG | % | 28 | | 25 | | 1.00 |
| Single valve | % | 44 | | 44 | | 1.00 |
| Valve + CABG | % | 11 | | 19 | | 0.65 |
| Other | % | 17 | | 13 | | 1.00 |
| ECC | (min) | 84.5 | (71.5-133) | 79.5 | (67.0-127) | 0.56 |
| ICU stay | (hours) | 22.5 | (18.8-25.0) | 23.0 | (18.5-25.3) | 0.77 |
| Ventilation time in ICU | (hours) | 3.0 | (1.0-4.3) | 2.5 | (1.0-4.8) | 0.96 |
| Inotropes in ICU | % | 0 | | 6 | | 0.47 |

Data are presented as percentages, medians (25th-75th percentile) or means ±SD.
Abbreviations: BMI = Body mass index; eGFR = Estimated glomerular filtration rate (Cockcroft-Gault equation); IDDM = Insulin dependent diabetes mellitus; COPD = Chronic obstructive pulmonary disease; LVEF = Left ventricular ejection fraction; CABG = Coronary artery bypass grafting; ECC = Extracorporeal circulation; ICU = Intensive care unit

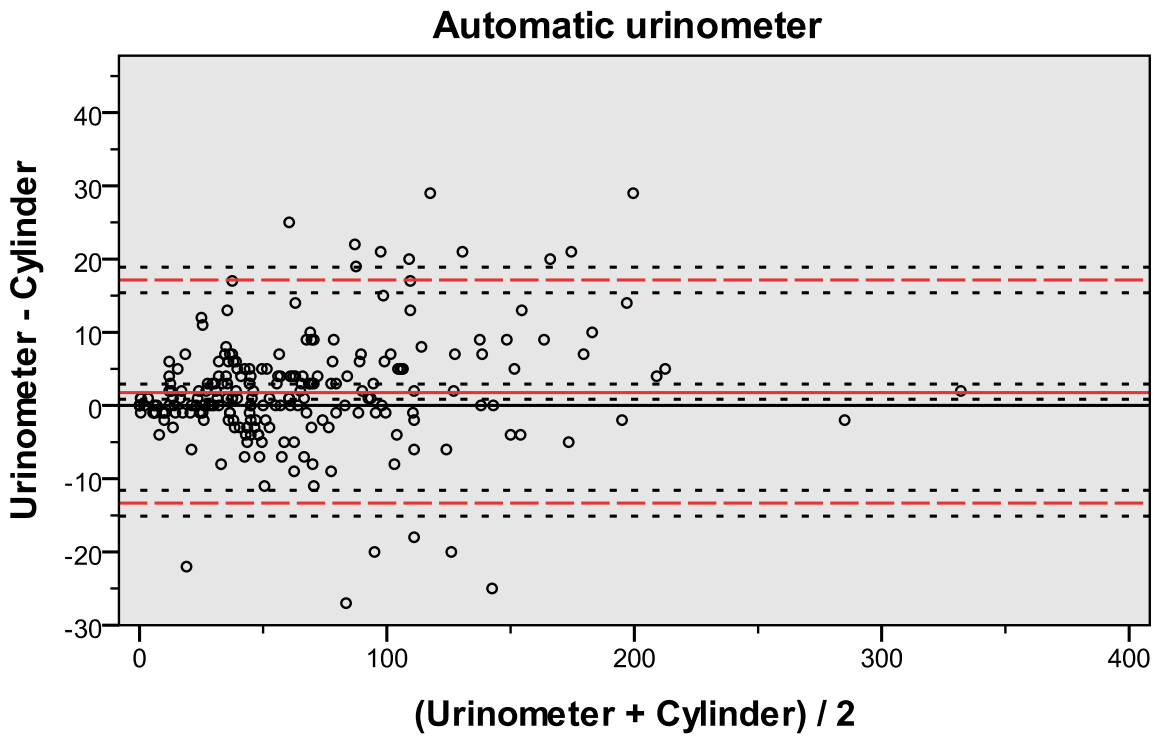
We excluded 5.6% (13/233) of the measurements in the AU group from analysis, almost completely because of inappropriate positioning of the AU, e.g. after the patient had changed position from the hospital bed to a chair. The median ($25^{th}$-$75^{th}$ percentile) number of UO measurements for each patient was akin for both groups with 10.5 (9.0-16.3) for the AU and 12.5 (8.3-14.8) for the MU. We paired every measurement with a reference

measurement performed with the measuring cylinder. The mean of the cylinder measurements was 65 mL in the AU group and 96 mL in the MU group. Bland-Altman calculations (**Table 4**) and plots (**Figure 17**) displayed a mean bias of +1.9 mL for the AU and +5.3 mL for the MU ($p<0.0001$).

**TABLE 4**. Performance parameters of the automatic urinometer (AU) and the manual urinometer (MU) measured in milliliters (mL).

| Urinometer parameters (mL) | | | | Upper LOA | | | Bias | | | Lower LOA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Urinometer | | *n* | *SD* | *SE* | *CI +* | $\bar{x}$ | *CI -* | *CI +* | $\bar{x}$ | *CI -* | *CI +* | $\bar{x}$ | *CI -* |
| AU | All | 220 | 7.7 | 0.5 | +18.9 | +17.1 | +15.4 | +2.9 | +1.9 | +0.9 | -11.6 | -13.3 | -15.1 |
| | <100mL | 176 | 6.4 | 0.5 | +15.4 | +13.8 | +12.2 | +2.2 | +1.3 | +0.3 | -9.7 | -11.3 | -12.9 |
| | ≥100mL | 44 | 11 | 1.7 | +33.5 | +27.6 | +21.6 | +8.0 | +4.5 | +1.0 | -12.6 | -18.6 | -24.5 |
| MU | All | 188 | 8.4 | 0.6 | +23.9 | +21.8 | +19.8 | +6.5 | +5.3 | +4.1 | -9.2 | -11.3 | -13.4 |
| | <100mL | 124 | 4.5 | 0.4 | +13.1 | +11.6 | +10.4 | +3.6 | +2.8 | +2.0 | -4.8 | -6.2 | -7.6 |
| | ≥100mL | 64 | 12 | 1.4 | +38.1 | +33.1 | +28.2 | +13 | +10 | +7.2 | -8.0 | -12.9 | -17.9 |

For each urinometer, data are presented for all measurements combined, as well as subdivided at a volume of < or ≥100mL.
Abbreviations: AU = Automatic urinometer; LOA = Limit of agreement; MU = Manual urinometer; n = Number of measurements; SD = Standard deviation; SE = Standard error; CI = Confidence interval; $\bar{x}$ = Mean



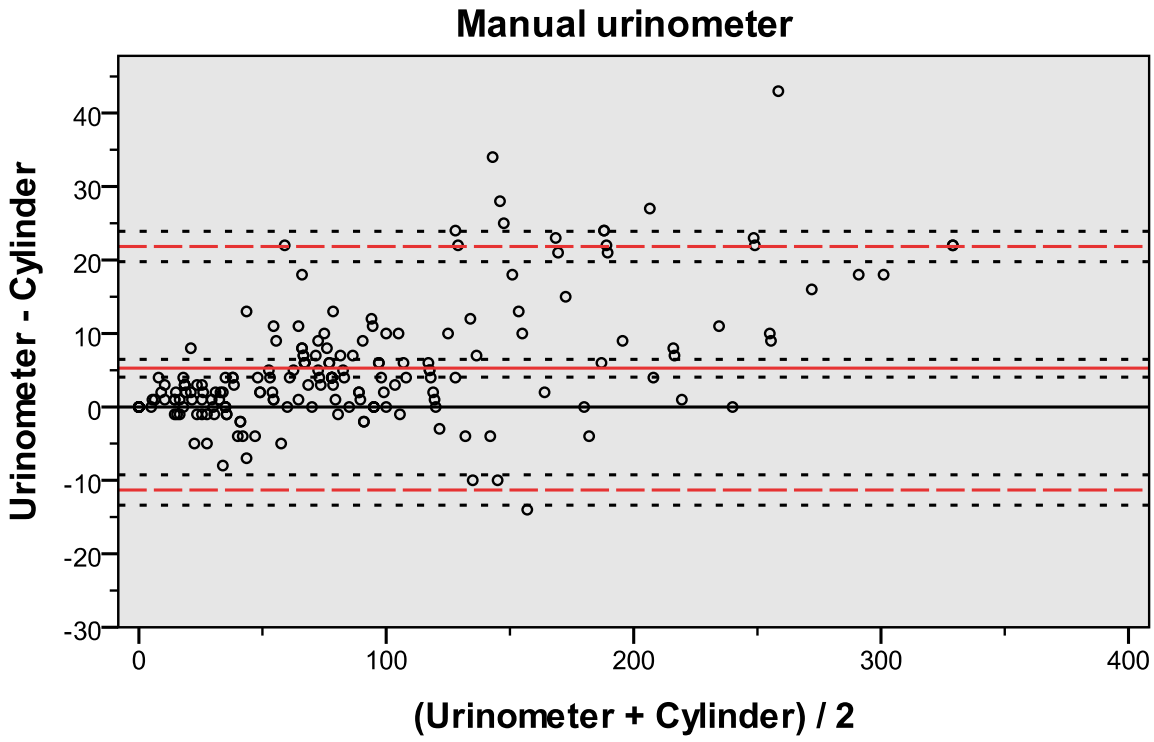



**Figure 17.** Bland-Altman plots of the agreement of each urinometer. Volumes are in milliliters (mL). In each plot the red lines depict (from above): Upper 95% limit of agreement; Mean bias; Lower 95% limit of agreement. Dotted lines depict confidence intervals of each parameter. Three measurements with x-axis volumes > 400 mL were omitted for the purpose of visibility.

The SD was 7.7 mL and 8.4 mL (p=0.108), respectively, presented by 95% limits of agreement intervals placed at ±15.2 mL and ±16.6 mL from the mean. The mean relative percentage deviation of the urinometers compared with their paired cylinder measurements were ±12.8% for the AU and ±12.7% for the MU (p=0.94). The scatter of the AU did not show any obvious change in bias with increasing urine volumes (**Figure 17**), and displayed estimated normality when mapped in a histogram (**Figure 18**). The MU scatter presented a propensity of a greater positive bias with rising UO (**Figure 17**), and depicted an inclination to skewness with a greater and longer tail to positive bias when mapped in a histogram (**Figure 18**).

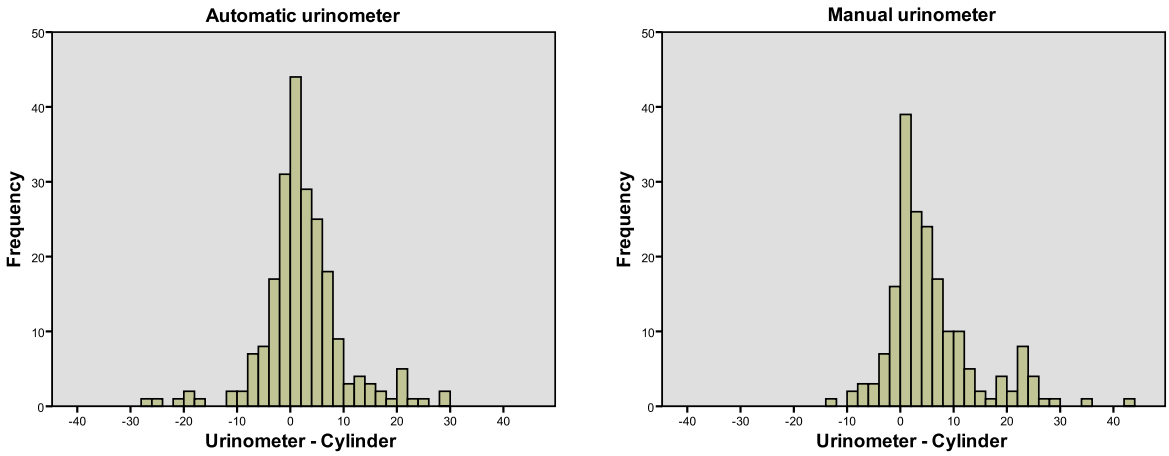



**Figure 18**. Histograms of the agreement of each urinometer. Volumes are in milliliters (mL). Each bar represents an increment of 2 mL. A positive value characterizes an overestimation by the urinometer compared with a reference measurement by the measuring cylinder.

Of the 408 measurements, 146 (36%) were recognized as an UO of <40 mL/h by either the used urinometer or the reference measurement. In this group of small urine volumes, the AU had a sensitivity of 90%, a specificity of 99%, a positive predictive value of 97% and a negative predictive value of 94%. The MU had a sensitivity of 98%, a specificity of 96%, a positive predictive value of 92% and a negative predictive value of 99% (**Table 5**).

**Table 5.** Evaluation of diagnostics of urine output <40mL/h. The automatic urinometer (AU) and the manual urinometer (MU) are compared with the measuring cylinder (gold standard).

| **Urinometer** | **AU** | **MU** |
|---|---|---|
| Sensitivity (%) | 90.4 | 98.2 |
| Specificity (%) | 98.5 | 96.2 |
| Positive predictive value (%) | 97.4 | 91.7 |
| Negative predictive value (%) | 94.4 | 99.2 |

We measured the duration between two sequential measurements and computed the absolute difference from exactly a full hour (n=108). The mean time-based variation of the MU was ± 7.4 minutes (±12.4%), 95% limits of agreement ±23.9 minutes (±39.8%), which should be compared with an absent temporal variation with the AU ($p<0.0001$).

All the 28 participating nurses filled out the questionnaires (**Table 6**) and 93 percent of them regarded the AU to be either easy or very easy to learn (question 1). The aggregate mean score of question 2 to 5 was 3.8 (SD ±0.9, $p<0.0001$ compared with mean=3), with 86% of the nursing staff judging the AU superior to the MU (personal mean>3) ($p<0.0001$). Altogether, 63% of the nurses were in favor of the AU, 5% were in favor of the MU and 32% graded the devices as equivalent.

**Table 6.** Staff opinion (n=28) of the automatic urinometer (AU) compared with the manual urinometer (MU**).**

| **Question** | | **Grading** | | | | |
|---|---|---|---|---|---|---|
| | | 5 | 4 | 3 | 2 | 1 |
| | | *Very easy* | *Easy* | *Fair* | *Not easy* | *Hard* |
| 1. | How easy was it to learn to use the automatic urinometer? | 39% | 54% | 7% | 0% | 0% |
| 2. | Was the collection of urine output data from the automatic urinometer easier compared with the manual urinometer? | 32% | 43% | 14% | 11% | 0% |
| | | *A lot less* | *Less* | *Same* | *More* | *Much more* |
| 3. | Did you feel that you had less contact with the urine bags with the automatic urinometer compared with the manual urinometer? | 36% | 25% | 39% | 0% | 0% |
| | | *Much more* | *More* | *Same* | *Less* | *Much less* |
| 4. | Do you think the reliability of the urine output data is higher with the automatic urinometer than with the manual urinometer? | 21% | 64% | 7% | 0% | 7% |
| 5. | Does using the automatic urinometer give you more time for other activities? | 0% | 32% | 68% | 0% | 0% |

## 4.2 STUDY II

We included 210 measurements, 127 with the AU and 83 with the MU from 12 patients (six AU, four MU, and two patients who used both devices) (**Table 7**). The mean weight of the children was 4.8 kg in both groups and the mean of the cylinder measurements was 18.7 mL in the AU group and 15.9 mL in the MU group, respectively (p=0.24).

**Table 7.** Characteristics and clinical variables of pediatric patients and number of urine output measurements on each patient by the automatic urinometer (AU) and the manual urinometer (MU).

| **Patient characteristics** | | | **Data at ICU admission** | | | | **ICU stay** | | **Urinometer recordings** | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | Sex | Kg | Reason for ICU admission | PDR (%) | Albumin (g/L) | Creatinine (µmol/L) | LOS | Time in respirator | AU | MU |
| 4 d | M | 3.6 | Post-op esophageal atresia | 1.4 | 22 | 81 | 3 d | 24 h | 10 | – |
| 4 d | M | 3.6 | Post-op diaphragmatic hernia | N/A | 21 | 97 | 4 d | N/A | – | 9 |
| 2 m | M | 3.7 | Hypoxic cerebral injury | 11.7 | 23 | 82 | 6 d | 5 d | 16 | – |
| 21 d | M | 3.9 | Upper respiratory tract infection | 1.8 | 23 | 28 | 3 d | 2 d | 5 | – |
| 3 d | M | 4.1 | Post-op esophageal atresia | 1.0 | 21 | 113 | 3 d | 2 d | 8 | 19 |
| 13 d | M | 4.2 | Post ECMO, meconium aspiration | 7.5 | 26 | 35 | N/A | N/A | – | 5 |
| 8 d | M | 4.3 | Post-op suspected meningocele | 1.1 | 24 | 47 | 3 d | 2 d | 3 | – |
| 3 m | F | 4.4 | Cerebral hemorrhage, seizures | 3.9 | 12 | 43 | 4 d | 4 d | 13 | – |
| 2.5 m | M | 5.2 | Post-op biliary atresia | 0.4 | 26 | 24 | 13 h | N/A | – | 7 |
| 3 m | M | 5.6 | Respiratory failure in VACTERL patient | 1.4 | 24 | 29 | 26 d | 20 d | 65 | 31 |
| 9 m | F | 6.0 | Bocha virus in premature child | 35.8 | 38 | 25 | 10 d | N/A | – | 12 |
| 17 m | F | 9.0 | Bacterial meningitis | 2.2 | 21 | 13 | 24 h | N/A | 7 | – |

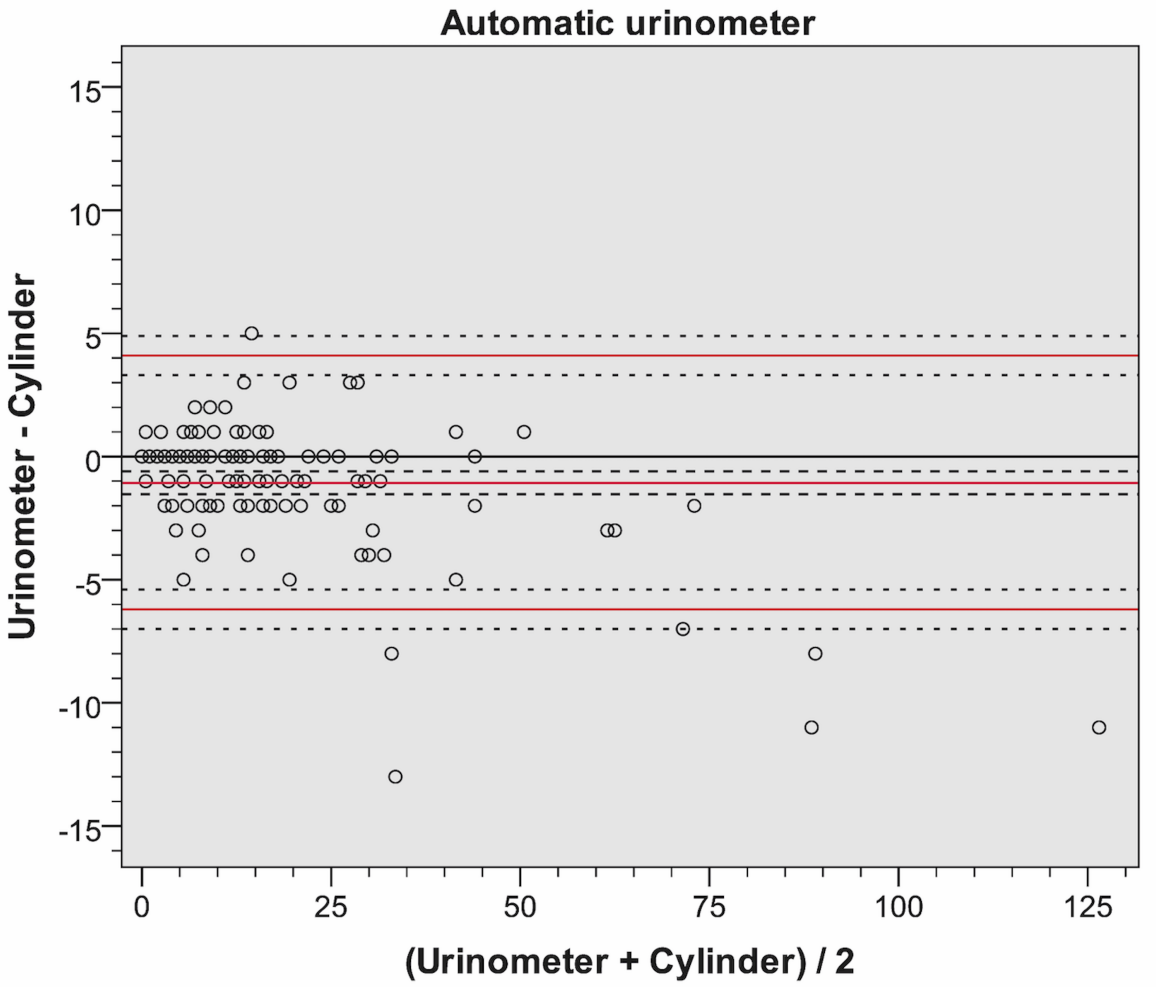
Abbreviations: d = days; F = female; h = hours; LOS = length of stay; M = male, m = months; PDR = predicted death rate. VACTERL = vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, limb abnormalities

The absolute mean bias was −1.1 mL (CI: −0.6; −1.5) and −0.6 mL (CI: ±0.0; −1.2) for the AU the MU, respectively (p=0.21). The SD:s were 2.6 mL and 2.8 mL, respectively (**Table 8**). The spread of the MU measurements did not show any obvious change of bias with increasing urine volume, while the AU had a small propensity to a larger negative bias with rising urine volume (**Figure 19**). When depicted in a histogram, the deviations from the reference cylinder values of the AU and the MU displayed near normality (**Figure 20**).

**Table 8.** Performance parameters of the automatic urinometer (AU) and the manual urinometer (MU) measured in milliliters (mL).

| **Urinometer parameters** | | | | **Upper LOA** | | | **Bias** | | | **Lower LOA** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***n*** | ***SD*** | ***SE*** | ***CI +*** | $\bar{x}$ | ***CI -*** | ***CI +*** | $\bar{x}$ | ***CI -*** | ***CI +*** | $\bar{x}$ | ***CI -*** |
| AU | 127 | 2.6 | 0.2 | +4.9 | +4.1 | +3.3 | -0.6 | -1.1 | -1.5 | -5.4 | -6.2 | -7.0 |
| MU | 83 | 2.8 | 0.3 | +6.1 | +5.0 | +4.0 | ±0.0 | -0.6 | -1.2 | -5.1 | -6.2 | -7.2 |
| | | p=0.96 | | | | | | p=0.21 | | | | |

Abbreviations: LOA = Limit of agreement; n = Number of measurements; SD = Standard deviation; SE = Standard error; CI = Confidence interval; $\bar{x}$ = Mean


Automatic urinometer
Urinometer - Cylinder
15
10
5
0
-5
-10
-15
0
25
50
75
100
125
(Urinometer + Cylinder) / 2

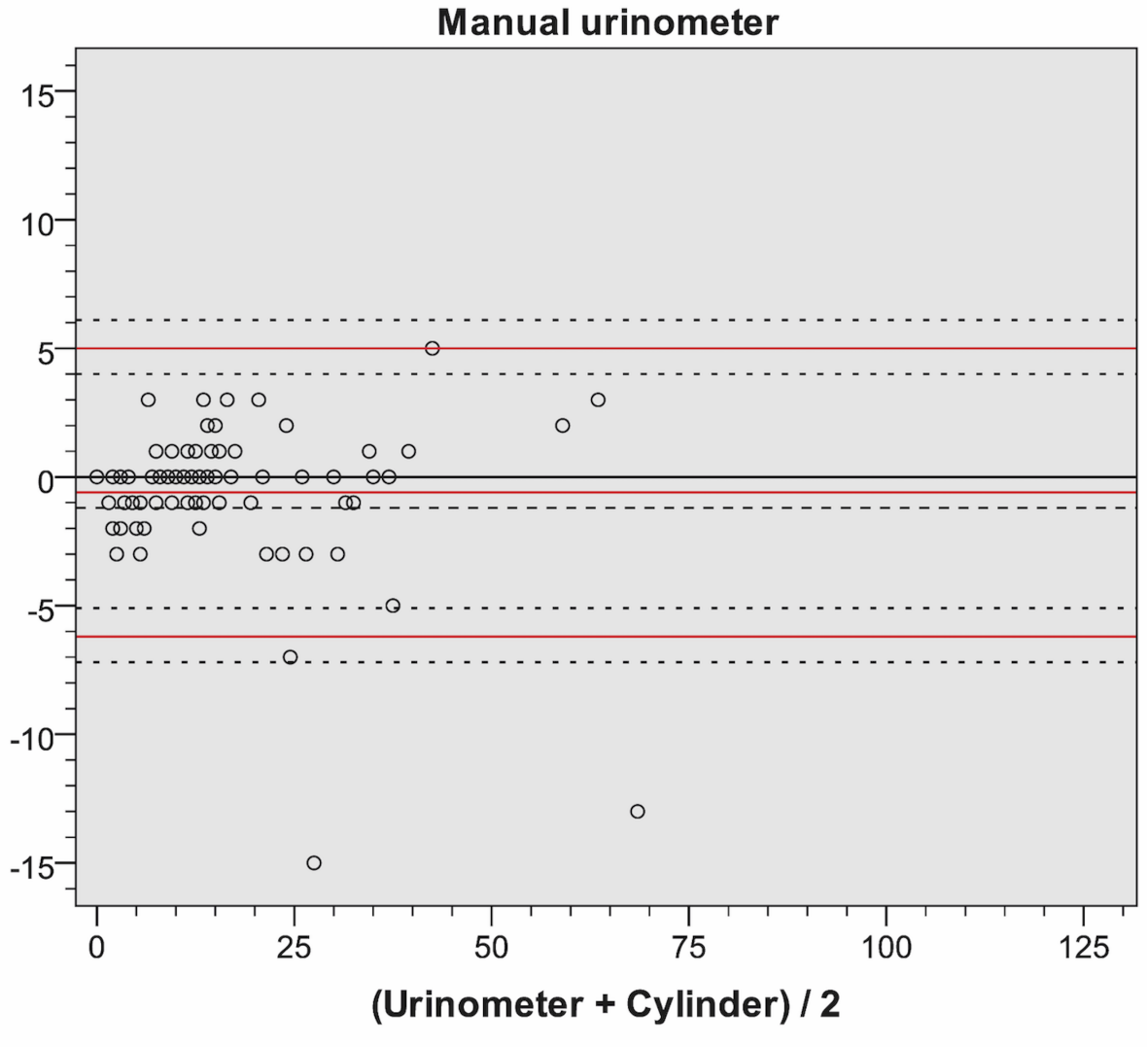



**Figure 19.** Bland-Altman plots of the agreement of both urinometer. Volumes are given in milliliters. In each plot, the red lines depict (from above): upper 95% limit of agreement; mean bias; lower 95% limit of agreement. Dotted lines depict confidence intervals of each parameter.

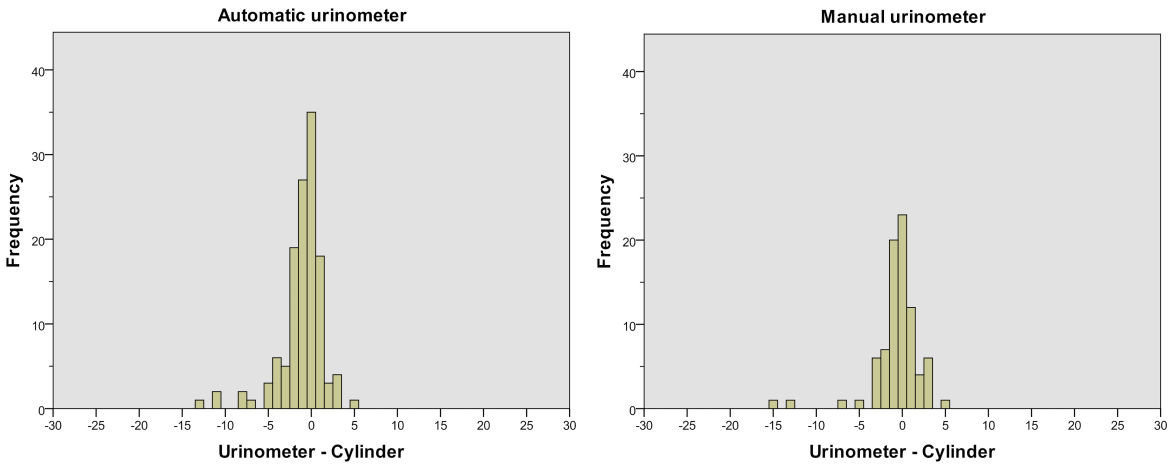



**Figure 20.** Histograms of the agreement between each urinometer. Volumes are given in milliliters (mL). Each bar represents an increment of 2 mL. A positive value represents an overestimation of the urinometer compared with a reference measurement by the measuring cylinder.

18 nurses included in the study filled out the evaluation questionnaire (**Table 9**). 94% of the nurses regarded the AU very easy or easy to learn (Question 1). For Questions 2-5 the aggregate mean score was 4.1 (SD ±0.8, $p<0.0001$ when compared with a mean of 3). Approximately 79% of the nurses preferred the AU, 1% preferred the MU and 19% graded the two urinometers as equivalent.

**Table 9.** Staff opinion (n=18) of the automatic urinometer compared with the manual urinometer.

| **Question** | | **Grading** | | | | |
|---|---|---|---|---|---|---|
| | | 5 | 4 | 3 | 2 | 1 |
| | | *Very easy* | *Easy* | *Fair* | *Not easy* | *Hard* |
| 1. | How easy was it to learn to use the automatic urinometer? | 50% | 44% | 6% | 0% | 0% |
| 2. | Was the collection of urine output data from the automatic urinometer easier compared with the manual urinometer? | 50% | 44% | 0% | 6% | 0% |
| | | *A lot less* | *Less* | *Same* | *More* | *Much more* |
| 3. | Did you feel that you had less contact with the urine bags with the automatic urinometer compared with the manual urinometer? | 56% | 22% | 22% | 0% | 0% |
| | | *Much more* | *More* | *Same* | *Less* | *Much less* |
| 4. | Do you think the reliability of the urine output data is higher with the automatic urinometer than with the manual urinometer? | 11% | 61% | 28% | 0% | 0% |
| 5. | Does using the automatic urinometer give you more time for other activities? | 16% | 56% | 28% | 0% | 0% |

## 4.3 STUDY III

### 4.3.1 Biofilm formed on polypropylene compared with polystyrene plastic

In **Study III** we studied if the type of plastic influenced biofilm formation, and compared polypropylene plastic, used in the AU, with polystyrene. We found that the wild type *E. coli* #12 strain and *C. albicans* formed significantly less biofilm when cultured on polypropylene compared with polystyrene plastic (**Figure 21A** and **Figure 21B**; $p<0.05$ and $p<0.01$).

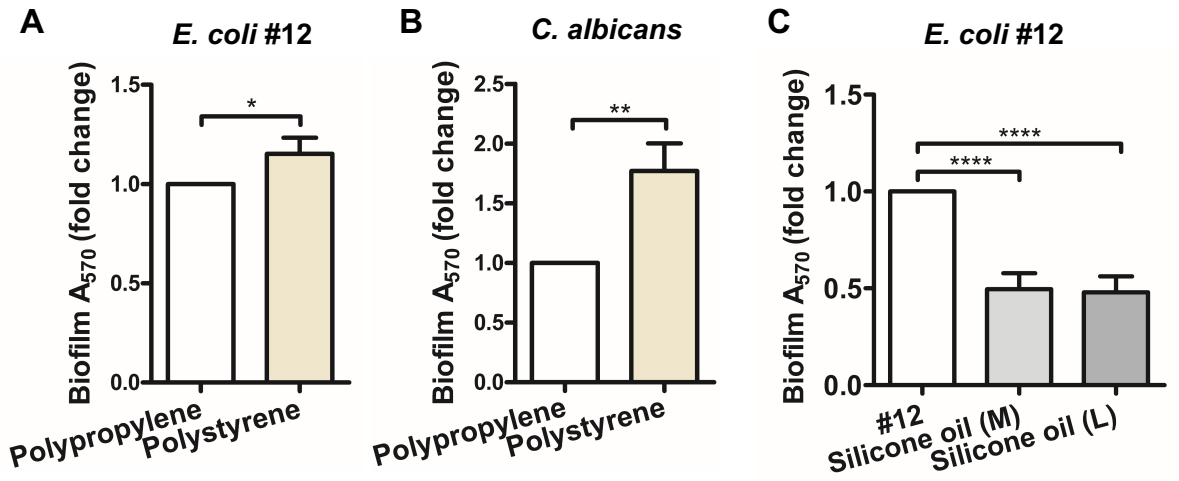



**Figure 21.** Polypropylene plastic prevented formation of biofilm better than polystyrene, whereas viscosity of silicone oil had no impact. The effect of polypropylene compared with polystyrene on biofilm formation was analyzed for *E. coli* #12 (A) and *C. albicans* (B). Both low and medium viscosity silicone oils significantly decreased biofilm formation of *E. coli* #12 on a polypropylene plate (C). L = Low viscosity. M = Medium viscosity. P-value: * ≤0.05; ** ≤0.01; *** ≤0.001; **** ≤0.0001.

### 4.3.2 Viscosity of silicone oil did not affect prevention of biofilm

Both low and medium viscosity silicone oil prevented biofilm formation similarly, as depicted in **Figure 21C** for *E. coli* #12 ($p<0.0001$). We concentrated on the medium viscosity silicone oil, as the water-soluble capsule in the antechamber of the AU contains this specific oil. However, we did test all bacteria and *C. albicans* with both silicone oils and found comparable results (**Figure 23** and **Figure 25**).

### 4.3.3 Medium viscosity silicone oil decreased biofilm formation

To explore if silicone oil may prevent the formation of new biofilm by microorganisms, we examined the Gram-negative bacteria *E. coli* CFT073*, P. aeruginosa, Prot. mirabilis* and *K. pneumoniae*, known to form biofilm (95-97). We found that medium viscosity silicone oil significantly inhibited biofilm production in all the tested bacteria (**Figure 22A-D;** $p<0.01$ and $p<0.001$).

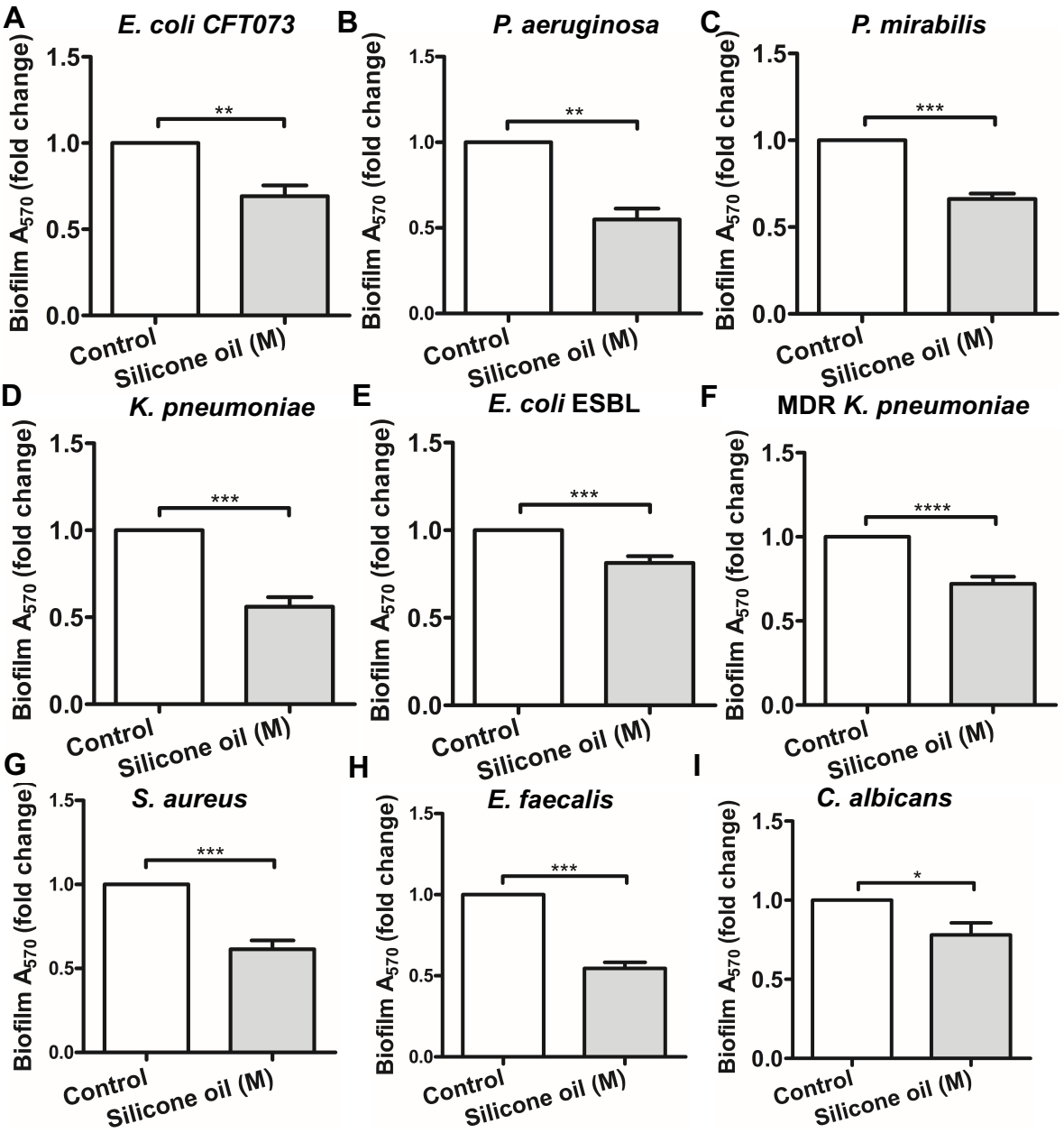



**Figure 22.** Medium silicone oil significantly reduced biofilm formation from common pathogens. This effect was demonstrated on *E. coli* CFT073 (A), *P. aeruginosa* (B)*, Prot. mirabilis* (C)*, K. pneumoniae* (D), ESBL *E. coli* (E), MDR *K. pneumoniae* (F), *S. aureus* (G), *Ent. faecalis* (H) and *C. albicans* (I). M = Medium viscosity.
P-value: * ≤0.05; ** ≤0.01; *** ≤0.001; **** ≤0.0001.

Furthermore, medium viscosity silicone oil also decreased biofilm formation of an ESBL-producing *E. coli* strain (**Figure 22E**; $p<0.001$) and a multidrug resistant *K. pneumoniae* strain (**Figure 22F**; $p<0.0001$), giving additional support that silicone oil generally inhibits bacterial biofilm formation. In addition, silicone oil significantly decreased biofilm by two Gram-positive bacteria, *S. aureus* and *Ent. faecalis* (**Figure 22G**; $p<0.001$ and **Figure 22H**, $p<0.001$). Finally, silicone oil significantly inhibited biofilm formation by *C. albicans* (**Figure 22I**, $p<0.05$).

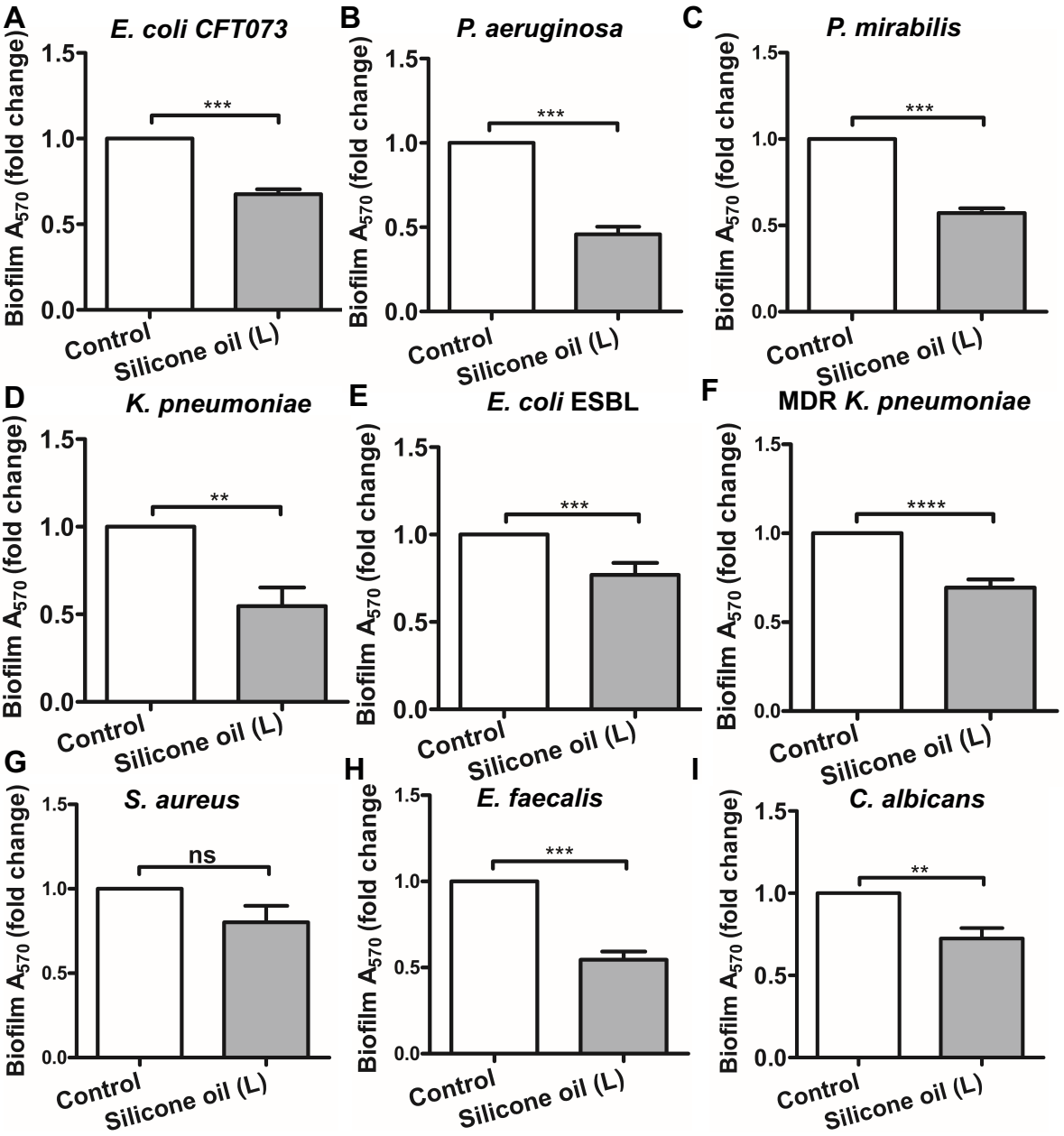



**Figure 23.** Low silicone oil significantly reduced biofilm from common pathogens like *E. coli* CFT073 (A), *P. aeruginosa* (B)*, Prot. mirabilis* (C)*, K. pneumoniae* (D), ESBL *E. coli* (E), MDR *K. pneumoniae* (F), *Ent. faecalis* (H) and *C. albicans* (I), whereas we did not find a significant impact on *S. aureus* (G). L = Low viscosity.
P-value: * ≤0.05; ** ≤0.01; *** ≤0.001; **** ≤0.0001.

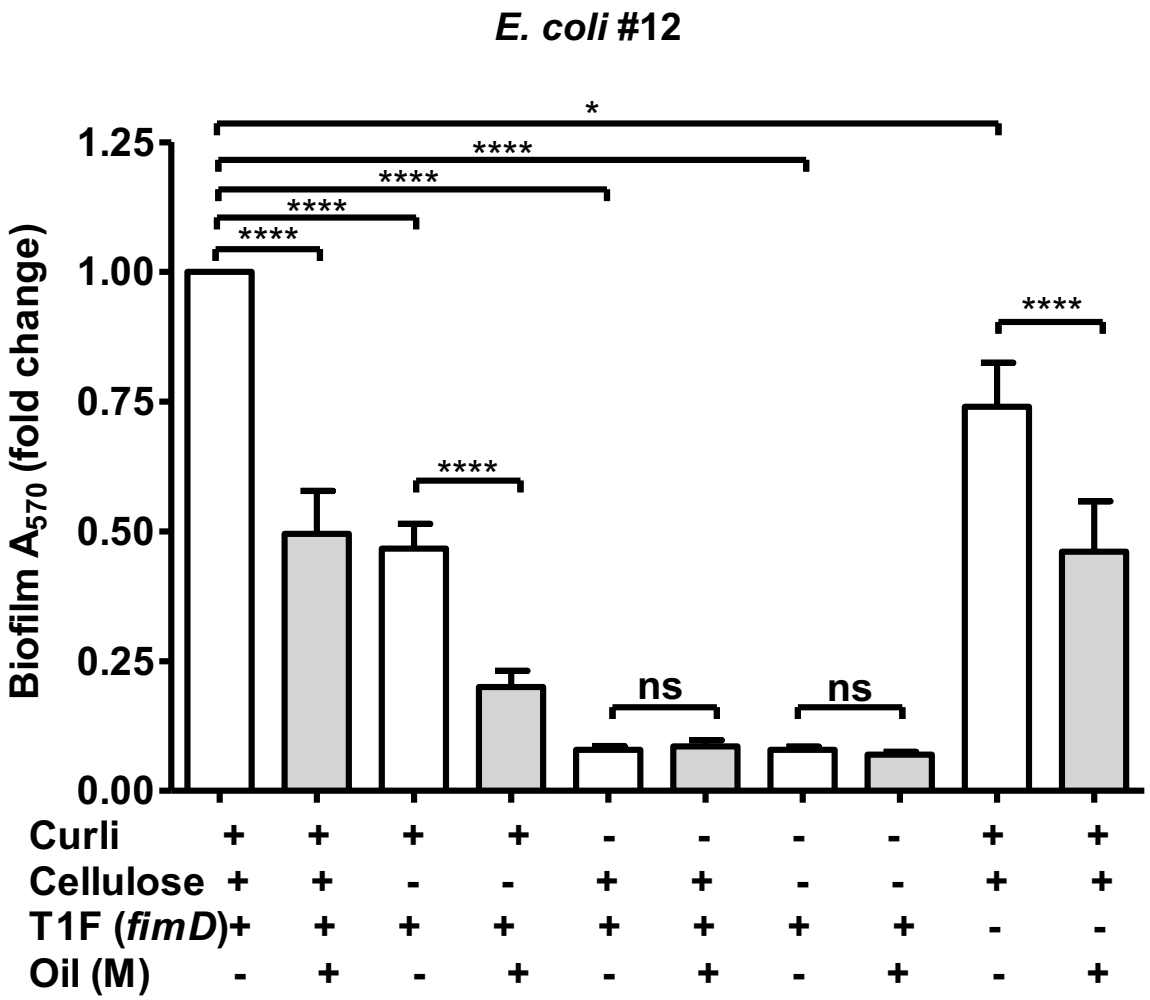



**Figure 24.** Medium viscosity silicone oil affects curli fimbriae. There was a significant reduction of biofilm formation of *E. coli #12, E. coli* (curli+/cellulose−/type 1 fimbriae (*fim D*+)) and *E. coli* (curli+/cellulose+/type 1 fimbriae (*fim D*−)). Medium viscosity silicone oil did not have a significant effect on biofilm formation of *E. coli* (curli−/cellulose+/type 1 fimbriae (*fim D*+)) nor on that of the double knock-out *E. coli* (curli−/cellulose/type 1 fimbriae (*fim D*+)). T1F = Type 1 fimbriae. M = Medium viscosity.
P-value: * ≤0.05; ** ≤0.01; *** ≤0.001; **** ≤0.0001.

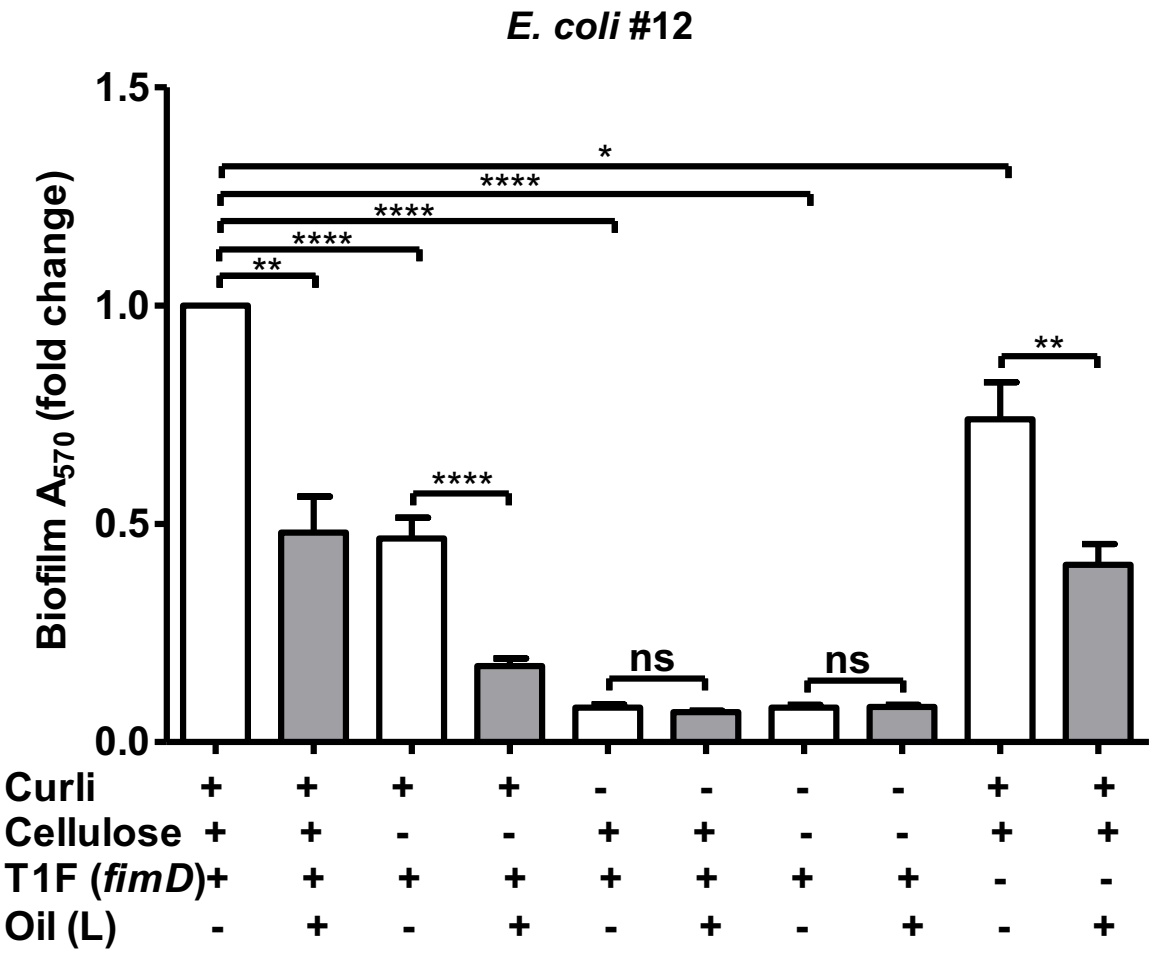



**Figure 25.** Low viscosity silicone oil targets curli fimbriae. A significant reduction of biofilm from *E. coli #12, E. coli* (curli+/cellulose−/type 1 fimbriae (*fim D*+)) and *E. coli* (curli+/cellulose+/type 1 fimbriae (*fim D*−)) was noticed. No significant effect of the oil was seen on *E. coli* (curli-/cellulose+/type 1 fimbriae (*fim D*+)) nor on the double knock-out *E. coli* (curli−/cellulose−/type 1 fimbriae (*fim D*+)). The effect on *E. coli* isogenic strains were studied for both medium (**Figure 24**) and low viscosity silicone oil at the same time. Thus, the untreated controls were the same. T1F = Type 1 fimbriae. L = Low viscosity.
P-value: * ≤0.05; ** ≤0.01; *** ≤0.001; **** ≤0.0001.

### 4.3.4 Curli fimbriae is a major target for biofilm formation by Gram-negative bacteria

We used the isogenic mutants of *E. coli* #12 lacking curli, cellulose or type 1 fimbriae (*fim D*), alone or in combination, to evaluate to what degree different bacterial virulence factors influenced biofilm formation on polypropylene plastic. The combination of all three virulence factors promoted most new biofilm formation, whereas absence of type 1 fimbriae significantly decreased biofilm formation. *E. coli* expressing type 1 fimbriae with or without cellulose only added to a low degree to biofilm formation (**Figure 24** and **Figure 25**)**.** Silicone oil did not influence the growth rate of *E. coli* #12, *S. aureus* nor *C. albicans* (**Figure 26**). Thus, we postulated that silicone oil has a direct impact on at least one of these main biofilm mechanisms (87).

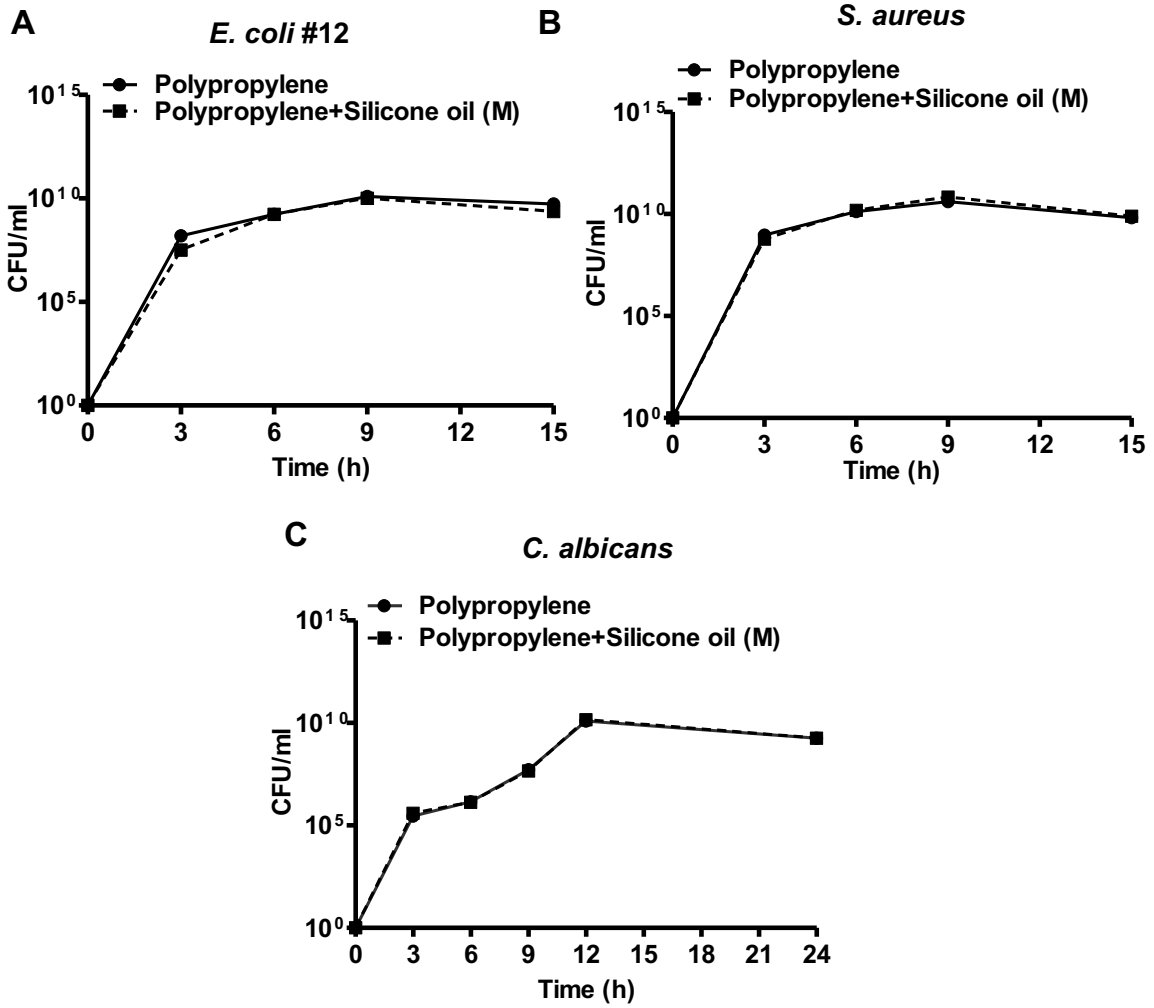



**Figure 26**. Number of colony forming units (CFU) analyzed using viable count. Increase in CFU is depicted with and without silicone oil in polypropylene tubes demonstrated with a log scale/mL for (A) *E. coli* #12, (B) *S. aureus* and (C) *C. albicans*. Control, bacteria only, cultured in polypropylene tubes: Black line; Bacteria grown with medium viscosity silicone oil in polypropylene tubes: Dotted line.

When exposing the curli-expressing *E. coli* strains to medium viscosity silicone oil, we found a significant decrease in biofilm growth (**Figure 24** and **Figure 25**; $p<0.0001$), but this was not seen in isogenic curli deficient strains. Likewise, medium viscosity silicone oil did not alter biofilm production by type 1 fimbriae or cellulose. Overall, our results showed that medium viscosity silicone oil targeted curli fimbriae and decreased biofilm formation.

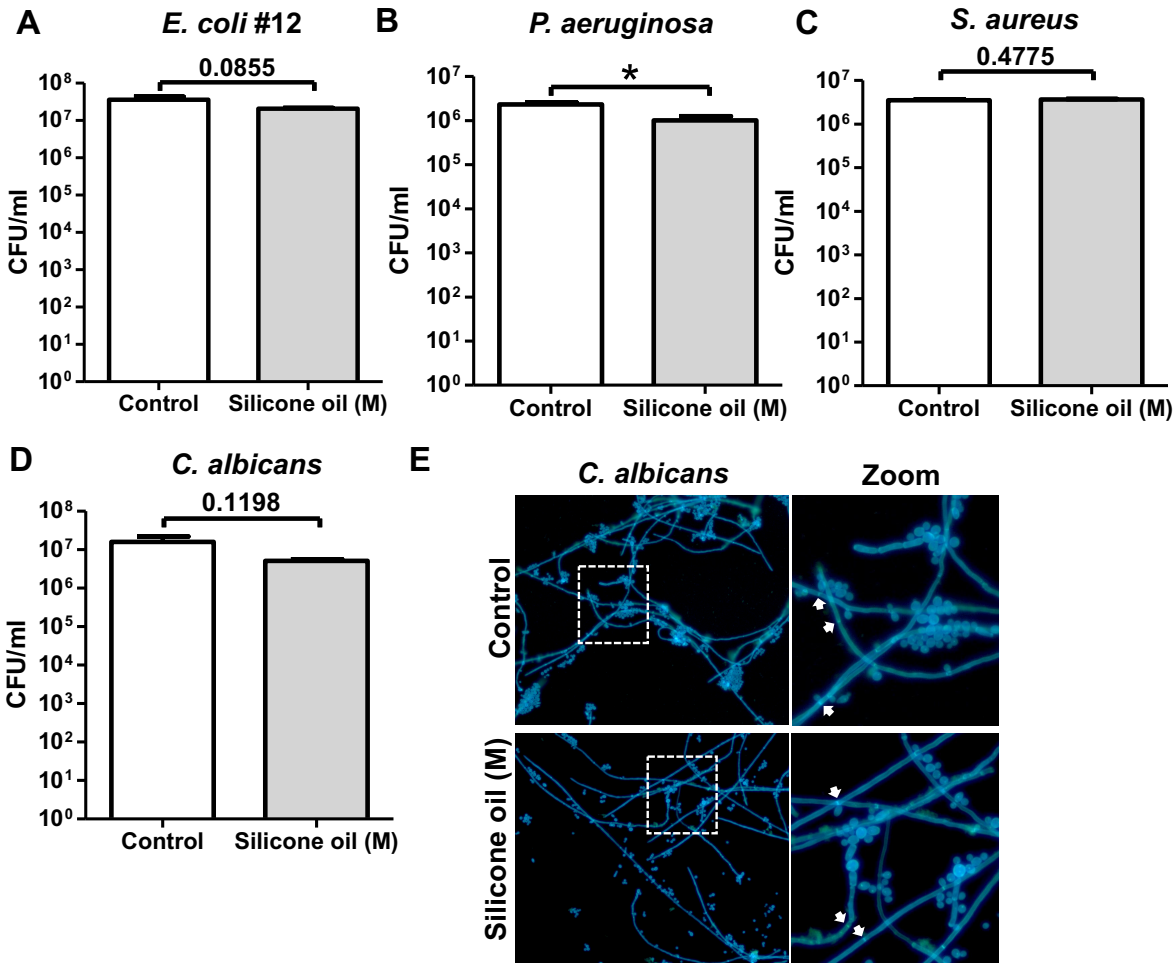



**Figure 27.** Medium viscosity silicone oil did not have a significant impact on the attachment of *E. coli* #12, *S. aureus* or *C. albicans* to polypropylene or on fungal hyphae. Nevertheless, *P. aeruginosa* adhered less to polypropylene ($p<0.05$). Number of colonies in mature biofilm after 72 hours of growth determined by viable count are depicted in log scale CFU/mL. (A) *E. coli #12* (B) *P. aeruginosa* (C) *S. aureus* (D) *C. albicans. C. albicans* staining was achieved from overnight growth in yeast-peptone-dextrose (YPD) broth in 30°C. (E; upper panel) *C. albicans* cultured in polypropylene tubes, (E; lower panel) *C. albicans* cultured in medium viscosity coated polypropylene tubes. We used Blankophor p to stain *C. albicans* (E; right panel). Septum are highlighted with white arrows. We did not notice differences in the fungal hyphae. P-value: * ≤0.05.

### 4.3.5 Mature biofilm induces less P. aeruginosa adhesion

We did not find any significant difference of viable count (CFU/mL) for *E. coli* #12, *S. aureus* and *C. albicans* in mature biofilm (**Figure 27A, C-D**). Nevertheless, *P. aeruginosa*, adhered to a lower extent to polypropylene ($p<0.05$) (**Figure 27B**).

### 4.3.6 Morphology of *C. albicans* hyphae after exposure to silicone oil

We did not observe any change in *C. albicans* hyphae after overnight exposure to medium viscosity silicone oil. Budding cells and septum were clearly seen with and without silicone oil (**Figure 27E**).

## 4.4 STUDY IV

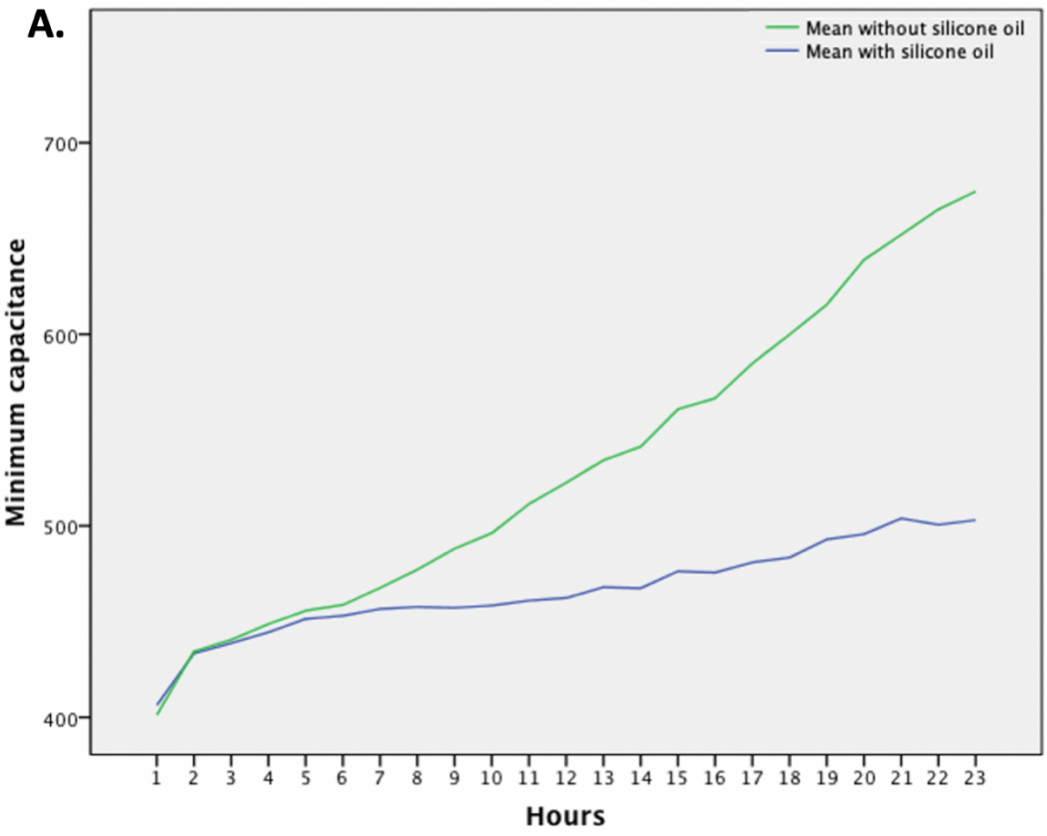
### 4.4.1 Effect on capacitance measurement by albumin

We analyzed 477 measurements without and 472 measurements with silicone oil, respectively. During the 24$^{th}$ hour, 29 measurements were excluded as readings were too few to extract a reliable average. Moreover, in a few cases the liquid container was emptied and the pump ran dry before the end of the 24$^{th}$ hour measurement period. The maximum capacitance value was 790 in the group without and 633 in the group with silicone oil, respectively. The mean increase in capacitance was 257±96 in the group without and 105±32 in the group with silicone oil, respectively. After ten hours of registration differences between the groups reached statistical significance ($p=0.011$, **Table 10**). The buildup of albumin coating over time is summarized in **Figure 28**, shown as the mean of the minimum capacitance, with and without silicone oil. The additional experiments with 0.3 g/L and 1.0 g/L albumin solution with 379 and 190 measurements, respectively, did not show significant differences in capacitance with and without silicone oil during the 23-hour time frame (data not shown).

**Table 10**. Capacitance parameters and change over 23 hours with albumin solution (3 g/L).

| | With silicone oil (n=472) | | | | | Without silicone oil (n=477) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Hour** | Min cap | Max cap | Median | Mean min cap | SD | Min cap | Max cap | Median | Mean min cap | SD | P-value (mean min cap) |
| **1** | 381 | 433 | 405 | 406 | 12 | 382 | 415 | 406 | 401 | 10 | 0.176 |
| **2** | 413 | 458 | 433 | 433 | 11 | 406 | 464 | 438 | 434 | 17 | 0.56 |
| **3** | 417 | 471 | 439 | 439 | 15 | 407 | 482 | 437 | 441 | 18 | 0.839 |
| **4** | 417 | 479 | 447 | 444 | 17 | 418 | 488 | 443 | 449 | 19 | 0.756 |
| **5** | 406 | 510 | 454 | 451 | 21 | 426 | 506 | 446 | 456 | 26 | 0.914 |
| **6** | 422 | 497 | 452 | 453 | 19 | 416 | 554 | 450 | 459 | 36 | 0.903 |
| **7** | 426 | 506 | 457 | 457 | 19 | 420 | 566 | 457 | 468 | 39 | 0.675 |
| **8** | 426 | 508 | 455 | 458 | 19 | 418 | 583 | 460 | 477 | 45 | 0.386 |
| **9** | 423 | 499 | 454 | 457 | 17 | 423 | 590 | 479 | 488 | 48 | 0.062 |
| **10** | 425 | 500 | 458 | 458 | 18 | 419 | 614 | 485 | 496 | 51 | 0.011 |
| **11** | 419 | 507 | 460 | 461 | 19 | 436 | 614 | 503 | 511 | 52 | <0.001 |
| **12** | 425 | 508 | 461 | 462 | 19 | 437 | 627 | 522 | 523 | 57 | <0.001 |
| **13** | 428 | 505 | 473 | 468 | 20 | 454 | 649 | 524 | 534 | 58 | <0.001 |
| **14** | 436 | 487 | 469 | 467 | 15 | 467 | 670 | 524 | 541 | 66 | <0.001 |
| **15** | 441 | 500 | 480 | 476 | 17 | 456 | 716 | 560 | 561 | 71 | <0.001 |
| **16** | 430 | 503 | 478 | 476 | 19 | 447 | 727 | 545 | 567 | 76 | <0.001 |
| **17** | 440 | 513 | 485 | 481 | 19 | 454 | 760 | 572 | 585 | 81 | <0.001 |
| **18** | 441 | 520 | 488 | 483 | 22 | 452 | 754 | 596 | 600 | 79 | <0.001 |
| **19** | 440 | 544 | 490 | 493 | 24 | 452 | 759 | 645 | 615 | 81 | <0.001 |
| **20** | 437 | 535 | 497 | 496 | 24 | 479 | 729 | 659 | 639 | 65 | <0.001 |
| **21** | 439 | 554 | 502 | 504 | 32 | 470 | 788 | 659 | 652 | 71 | <0.001 |
| **22** | 440 | 556 | 502 | 501 | 27 | 494 | 790 | 670 | 665 | 63 | <0.001 |
| **23** | 400 | 660 | 503 | 503 | 56 | 472 | 785 | 677 | 674 | 67 | <0.001 |

Abbreviations: Min cap = Minimum capacitance; Max cap = Maximum capacitance; SD = Standard deviation.



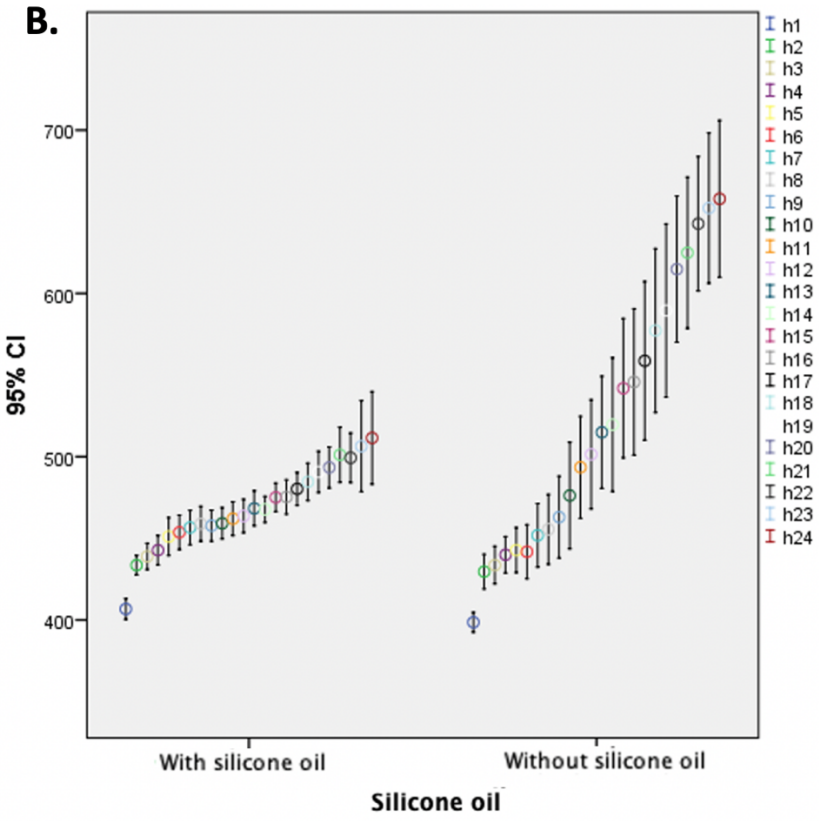




**Figure 28. A.** Mean capacitance measurements caused by an albumin solution during 23 hours coating for devices without (green) and with (blue) addition of silicone oil. B. Median, maximum and minimum capacitance values caused by an albumin solution for each hour with and without addition of silicone oil.

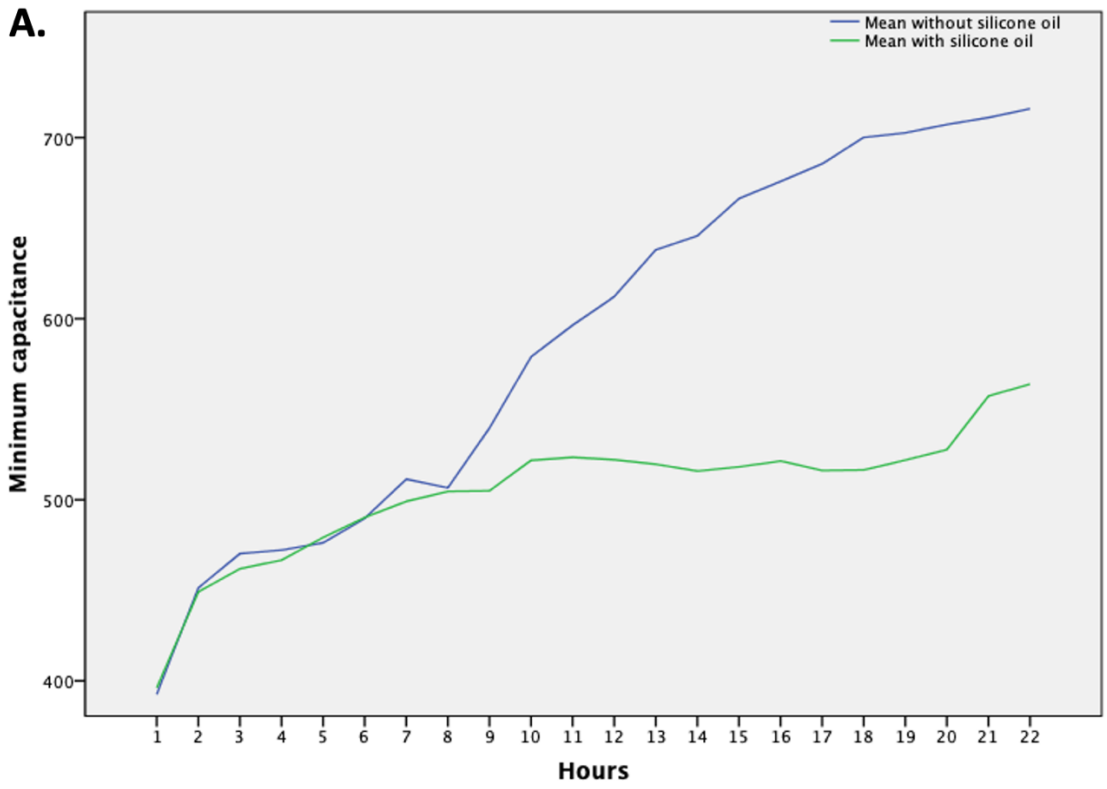
### 4.4.2 Effect on capacitance measurements by free hemoglobin

In total 484 measurements without and 414 with silicone oil were compared. The mean concentration of fHb in the prepared mixtures was 0.0141 g/L, ranging from 0.0056 g/L to 0.0173 g/L. The mean concentration in the group with silicone oil was 0.0113 g/L and 0.0125 g/L in the group without. The mean increase in capacitance was 190±174 with silicone oil and 324±78 without. A significant difference between the groups was seen after 20 hours and onwards (**Table 11**). The last two hours of measurements were excluded from the analysis as the readings during the 23$^{th}$ and 24$^{th}$ hours did not result in sufficient number of readings to test for differences. **Figure 29** depicts the mean increase in capacitance due to buildup of fHb coating over time in the group with and without silicone oil, respectively.

**Table 11**. Capacitance parameters and change over 22 hours with free hemoglobin solution (0.01 g/L).

| | **With silicone oil (n=414)** | | | | | **Without silicone oil (n=484)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Hour** | Min cap | Max cap | Median | Mean min cap | SD | Min cap | Max cap | Median | Mean min cap | SD | P-value (mean min cap) |
| **1** | 379 | 414 | 396 | 396 | 11 | 378 | 411 | 393 | 393 | 10 | 0.09 |
| **2** | 406 | 641 | 449 | 449 | 49 | 404 | 496 | 451 | 451 | 26 | 0.266 |
| **3** | 398 | 650 | 462 | 462 | 51 | 416 | 563 | 470 | 470 | 33 | 0.272 |
| **4** | 427 | 530 | 467 | 467 | 22 | 417 | 534 | 472 | 472 | 31 | 0.542 |
| **5** | 432 | 539 | 479 | 479 | 24 | 417 | 561 | 476 | 476 | 35 | 0.649 |
| **6** | 432 | 561 | 490 | 490 | 27 | 408 | 691 | 490 | 490 | 58 | 0.642 |
| **7** | 433 | 575 | 499 | 499 | 30 | 414 | 773 | 511 | 511 | 89 | 0.652 |
| **8** | 434 | 591 | 505 | 505 | 31 | 414 | 798 | 507 | 507 | 87 | 0.327 |
| **9** | 435 | 601 | 505 | 505 | 32 | 411 | 843 | 540 | 540 | 125 | 0.724 |
| **10** | 430 | 752 | 522 | 522 | 66 | 415 | 881 | 579 | 579 | 158 | 0.622 |
| **11** | 429 | 779 | 523 | 523 | 74 | 404 | 880 | 596 | 596 | 175 | 0.922 |
| **12** | 431 | 765 | 522 | 522 | 73 | 404 | 874 | 612 | 612 | 177 | 0.518 |
| **13** | 433 | 767 | 520 | 520 | 74 | 410 | 873 | 638 | 638 | 177 | 0.257 |
| **14** | 431 | 761 | 516 | 516 | 73 | 403 | 862 | 646 | 646 | 179 | 0.232 |
| **15** | 438 | 783 | 518 | 518 | 79 | 399 | 866 | 666 | 666 | 178 | 0.164 |
| **16** | 451 | 777 | 521 | 521 | 79 | 422 | 873 | 676 | 676 | 179 | 0.176 |
| **17** | 456 | 767 | 516 | 516 | 81 | 421 | 877 | 686 | 686 | 183 | 0.091 |
| **18** | 454 | 776 | 516 | 516 | 83 | 402 | 880 | 700 | 700 | 179 | 0.058 |
| **19** | 448 | 784 | 522 | 522 | 83 | 406 | 873 | 703 | 703 | 181 | 0.06 |
| **20** | 433 | 775 | 528 | 528 | 93 | 420 | 878 | 707 | 707 | 183 | 0.031 |
| **21** | 428 | 818 | 557 | 557 | 113 | 409 | 897 | 711 | 711 | 186 | 0.037 |
| **22** | 428 | 824 | 564 | 564 | 117 | 412 | 907 | 716 | 716 | 189 | 0.028 |

Abbreviations: Min cap = Minimum capacitance; Max cap = Maximum capacitance; SD = Standard deviation.



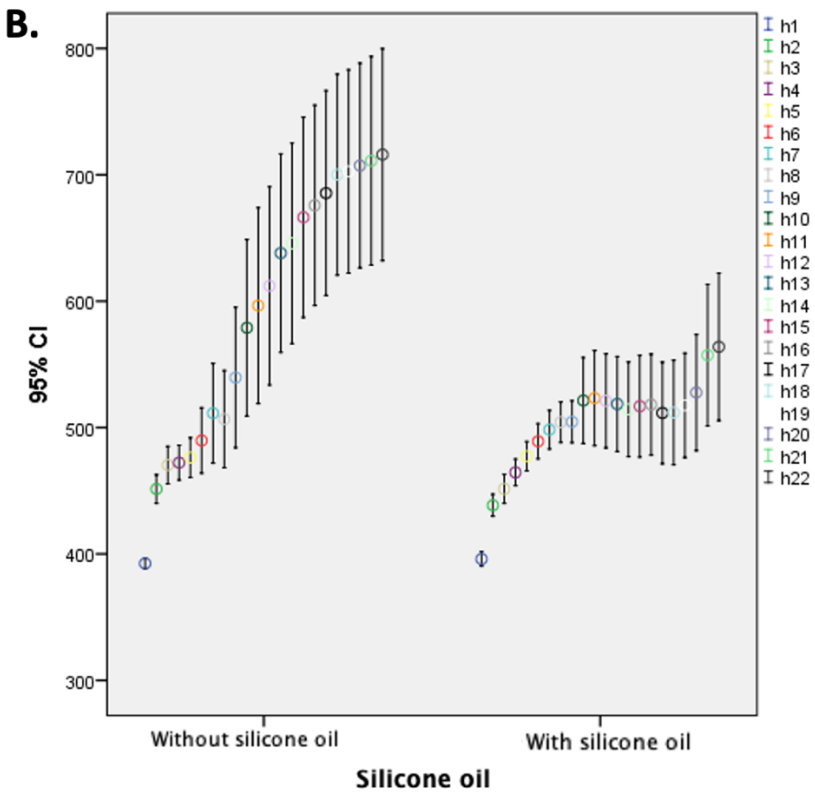




**Figure 29.** A. Mean capacitance measurements caused by a free hemoglobin solution during 22 hours coating for devices without (blue) and with (green) addition of silicone oil. B. Median, maximum and minimum capacitance values caused by a free hemoglobin solution for each hour with and without addition of silicone oil.

# 5 DISCUSSION

## 5.1 VALIDATION OF A CAPACITANCE-BASED AUTOMATIC URINOMETER

The best way to evaluate a new measuring device is to compare it with preferably the gold standard methods. Thus, in **Study I** and **II** we measured hourly measurement UO with the gold standard, a laboratory cylinder, and compared it with data acquired with the MU or the AU. The chosen study design permitted the comparison between the MU and the AU. The scientific scale is an alternative to the measuring cylinder and has two advantages as it is automatic and avoids visual assessment of UO. However, we chose not to use a scale as, based on our earlier studies (98), it is very sensitive to movements, which will easily distort measurements.

### 5.1.1 Evaluation of a new automatic urinometer in adults (Study I)

In brief, the new AU, using a capacitance measuring technique, was non-inferior to the MU, and scored significantly better regarding bias, temporal deviation and nursing staff judgement.

In **Study I,** the bias of the AU was significantly lower when compared with the MU. This corresponds to a 24-hour bias of 46 mL with the AU compared with 126 mL with the MU. Both these values may be good enough in clinical use, while a smaller bias is desirable. Single errors of this size would not change clinical therapy in our view. The level of precision did not disagree significantly between the AU and the MU. Both urinometers were in our view equivalently effective in detecting oliguria, although the number of patients were too few to give a conclusive answer in this respect.

As depicted in **Figure 17**, the bigger range of the dots at larger volumes in both plots indicates a worse precision at larger volumes. With the AU this may possibly be caused by a minor mismeasurement reoccurring for each siphon measurement, while with the MU this may be due to the inexact grading of the MU at large volumes and to an incorrect angle between the evaluator's eyes and the horizontal surface level of the urine in the MU. When a Bland-Altman plot deviates at larger means, the ratio of the difference and the mean can be drawn on the y-axis in place of the difference (99). We believed this method unsuitable, as this alternative would have transferred the error from high absolute divergences among large means to large relative divergences midst small means. Furthermore, we prefer absolute values clinically. The propensity towards skewness in the histogram of the MU

may imply that visual reading of the analogue scale of the MU largely overestimates the UO (**Figure 18**).

The possible question is what happens when the level of urine increases in the container (which would happen within 72 hours). However, only 16-18 mL of urine is collected in the measuring chamber of the AU before it is emptied automatically by a siphon mechanism into the collection bag. Although urine is emptied into the collection bag, oil will remain stuck onto the walls of the measuring chamber. Otherwise, the addition of the oil capsule would not have prevented the signal loss of the capacitance measurement that was registered and clinically observed before its introduction.

Hersch et al. used a similar study design when assessing an AU using a droplet counting technique (100). They also found their AU superior to the MU regarding bias, precision and user friendliness. When comparing their AU, based on droplet counting, with our tested AU which uses capacitance measurements, one should consider the following: The bias with the capacitance technique was +1.9 mL (SD ±7.7 mL), which is comparable with +0.08 mL (SD ± 14 mL) with the droplet counting technique. However, the bias of the MU in our study was +5.3 mL (SD ±8.4 mL) compared with the much higher value +13 mL (SD ±68 mL) in the study by Hersch et al. While the brand of the MU was identical in both studies, we do not know if the same models were applied, because Hersch et al. did not specify which model they utilized.

Goldman et al. compared an AU based on a temperature exchange electronic sensor technique (Clarity RMS™, RenalSense, Israel) with an MU using a scientific scale instead of a measuring cylinder as a gold standard. They found a mean UO difference for the AU-scale of −2.55 mL (95% CI, −4.3 to −0.8), and a mean difference for the MU-scale of +8.5 mL (95% CI, 5.4 to 11.7). Whereas the Clarity RMS™ slightly underestimated the measured volume, the capacitance-based AU Sippi® that we tested slightly overestimated the volume, but to a nearly identical degree (−2.55 mL vs. +1.9 mL). The bias of the MU was somewhat smaller in our study compared with their study (+5.3 mL vs. +8.5 mL). Notably, the measurement precision was considerably and significantly lower with the Clarity RMS™ (SD 25.8 mL) compared with the Sippi® that we tested (SD +7.7 mL).

#### *5.1.1.1 Temporal deviation of measurements with a manual urinometer*

One error which may occur when assessing UO is temporal deviation of measurements that additionally may lower the precision of the MU. Conversely, this error is intrinsically circumvented with the AU using its built-in precision clock. In **Study I**, the mean time-

based variation of the MU was ±7.4 minutes, compared with no temporal variation with the AU ($p<0.0001$). This is equivalent to a mean time error of ±12.4% and similar to the 16% found by Kramer et al. in manual monitoring UO in a standard ICU (24). In our view, this type of error may have clinical consequences.

### 5.1.2 Evaluation of a new automatic urinometer in children (Study II)

In **Study II** we investigated the capacitance-based AU in children, whereby we specifically adapted the AU by using a double-lumen tube instead of the normal single-lumen tube between the patient´s urinary catheter and the AU measuring device without changing the type or length of the tube. We did this for two reasons. Firstly, we kept both setups as similar as possible excluding for the measuring component, to improve the transit of small urine volumes. When a long single-lumen tube is used, small volumes of urine may get trapped without getting to the measuring chamber because of a negative pressure. Secondly, with a double-lumen tube, large urine volumes may flood the measuring chamber too quickly, when pressure differences between the two tubes are even out, resulting in miscalculation.

In **Study II**, the bias of the AU when extrapolated for 24 hours was 26.4 mL versus 14.4 mL for the MU group. Such daily biases are in our view acceptable in clinical practice and should likely not change clinical decisions. Largely, the AU underestimated UO somewhat, opposing the results in **Study I**. Conceivably, the double-lumen tube induced a too fast urine flow for the AU sensors when very high volumes occurred, depicted by a negative bias in the weightiest children with large urine volumes.

To our knowledge, this is the first and only published study evaluating an AU among pediatric patients.

### 5.1.3 Nursing staff´s evaluation of a manual versus an automatic urinometer

A prerequisite to get a new device accepted in clinical routine is that the staff get the necessary theoretical and practical training education. Thus, the staff's judgment of the new device after the training is of paramount significance. Both **Study I** and **II** began with a 15 minutes theoretical summary of the device including its handling, where after the nursing staff used the device during 3 days. Finally, the nursing staff filled out the questionnaire about the devices.

In **Study I**, the AU, while indeed at large preferred by the staff, did not score extraordinarily much better than the MU (**Table 6**). This is plausible because the AU device

at the time of the study did not automatically transfer UO data to the patient data management system. One may expect that the use of automatic data transfer would have increased the ratings of questions 2, 3 and 5, indicating an additional increase in staff satisfaction with the AU.

Compared with the cardiothoracic ICU nursing staff's evaluation in **Study I** (**Table 6**), the pediatric ICU nursing staff (**Study II**) (**Table 9**) generally rated the AU slightly higher, except for question 4 about reliability of UO measurement. Here, 85% of the cardiothoracic ICU nursing staff graded the AU higher, compared with 72% for the pediatric ICU nursing staff. Interestingly though, 28% of the pediatric ICU nursing staff thought the reliability was the same between the AU and the MU, compared with 7% of the cardiothoracic nursing staff. Also, for question 5, 68% of the adult cardiothoracic ICU nursing staff compared with 28% of the pediatric ICU nursing staff considered that neither of the devices gave them more time for other activities. Interestingly, 72% of the pediatric ICU nursing staff considered that the AU rendered more or much more time for other activities. These differences between our two studies may have many explanations. The introduction and teaching of the new AU may have differed between the ICUs. It is of uttermost importance that all the staff is familiar with all the features of the AU before starting the study, otherwise this might take extra time and energy. The fact that the system at the time of the studies was not linked to PDMS and thus obliged the nurse to manually record the UO may also have played a role. The more positive view of the pediatric nursing staff regarding the use of the AU may also be due to the fact that the UO are lower in children and that the measuring procedure, including making sure that the urinary catheter and the tube between the catheter and the UO measuring device is not kinked, is easier. Although a majority of the nurses in both the adult and the pediatric ICU regarded that they had a lot less or less contact with the urine bag of the AU compared with the MU, a fair part of the participating nurses in both ICUs felt they had the same degree of contact with both the AU and the MU. Normally, regular physical contact should not be needed with the AU. However, in this case, it may be explained by the novelty of the product and the need of reassuring its functionality. The argument that the AU saves time for the staff was demonstrated in the evaluation for both the adult and pediatric ICU patient. The nursing staff's positive opinion of the AU in **Study I** and **II** are in line with the study by Hersch et al (23), where the nursing staff evaluated a droplet-based AU called Urinfo 2000, which, however, is no longer commercially available.

## 5.2 REDUCTION OF BIOFILM FORMATION

Biofilm is a vital part of many infections. It weakens the penetration of antimicrobials and obstructs the response of the intrinsic immune system (39, 101). There is a need to decrease biofilm formation as it may promote infection of medical equipment, including temporary urinary and central venous catheters, permanent pacemakers and prosthetic heart valves (102). Thus, new efficacious methods to prevent biofilm formation are sought for. In **Study III**, we found that the type of used plastic material affected the growth of biofilm. The least quantity of biofilm was produced when bacteria were cultured on silicone oil covered polypropylene plastic. Additionally, curli fimbriae of *E. coli* were recognized as the principal target of silicone oil.

### 5.2.1 Impact of plastic and silicone oil on microbial biofilm formation

In **Study III** we studied polypropylene plastic as this is the plastic used in the measuring chamber of the studied AU. Polypropylene plastic alone reduced biofilm formation by *E. coli* #12 and *C. albicans* more than polystyrene, an alternative plastic applied in many medical equipment and devices. This polypropylene effect is deemed to its relatively slippery surface (81, 82). Due to the non-polar and hydrophobic properties attributed to polypropylene, silicone oil will accumulate as droplets on the surface of polypropylene. Based on the chemical structure and an initial pilot study using Raman microscopy (unpublished data), some amount of the hydrophobic oil will attach to the polypropylene plastic surface, in the form of droplets, and in doing so exerts the long lasting clinically observed effect despite surfaces being intermittently exposed to air between each emptying of the measuring chamber. Clinical observations and **Study IV**, suggest that supplementing silicone oil to polypropylene plastic impedes biofilm formation, prolonging adequate function of the AU.

One also has to consider the biofilm formation at the liquid/air interface, where biofilms like pellicles are formed. This can be selectively advantageous for aerobic or facultative aerobic bacteria. Wang et al. have previously demonstrated that loss of O-antigen was associated with enhanced pellicle formation (103). In **Study III** we observed that biofilm was formed on the liquid/air interface. Moreover, we observed biofilm components like pellicles on the surface of the liquid. We carefully stained the biofilm at the liquid/air interface.

In **Study III**, we found that the low as well as the medium viscosity silicone oils equally prohibited biofilm formation by common bacteria, implying that the grade of the silicone

oil viscosity did not have a significant impact on biofilm formation. In our view, silicone oil has a direct effect on biofilm formation, as silicone oil did not affect the bacterial growth. Our data indicate that silicone oil affects the biofilm promoting structure curli, without or with only minor impact on type 1 fimbriae or cellulose. Moreover, our viable count data corroborates that the decrease of biofilm is due to inhibition of extracellular matrix production and in the case of *E. coli*, this seems to be mediated via curli fimbriae.

Fungi, including *C. albicans,* can similarly to bacteria form biofilm and initiate lower urinary tract infections (38). Our finding in **Study III** that both polypropylene and silicone oil significantly decreased biofilm formation expressed by *C. albicans* is of clinical importance. In fact, Odabasi and Mert reported that 22% of patients remaining more than one week in an ICU had candiduria that in turn could significantly be linked to increased mortality (104).

The new AU contains a silicone oil capsule, which is liquefied by urine, where after silicone oil covers the surface of the polypropylene plastic measuring chamber. In **Study III** we demonstrated that the polypropylene plastic silicone oil interaction significantly decreased biofilm formed by common bacteria and candida, whereby the function of the device was prolonged and possibly may reduce the risk of an ascending urinary infection.

In **Study III**, we chose to focus on the polypropylene plastic used in the actual device. The choice of polypropylene plastic originally was made after clinical observations that polypropylene in combination with silicone oil proved benefits in terms of functional duration of the device. We decided to include polystyrene primarily to investigate whether the plastic on its own, without oil, could impact the amount of biofilm formation. However, including a plastic that could serve as a "better" positive control would most probably have been relevant, both in order to study the underlying mechanisms and to identify the main target.

Silicone oil could potentially target other components of the bacteria and the bacterial biofilm, which we did not test in **Study III**. Instead, we focused on *E. coli*, being an important pathogen and the major biofilm components curli, cellulose and type 1 fimbriae. To elucidate the bacterial biofilm targets we created knock out strains, allowing us to investigate the impact of these components. In **Study III,** we were able to clearly demonstrate the impact of curli. However, *Klebsiella spp* have not been shown to be equipped with curli or cellulose (105) and other components are therefore relevant for the biofilm formation. This merits further investigation, but that was beyond the scope of **Study III**.

### 5.2.2 Impact of silicone oil on biofilm caused by albumin and free hemoglobin

The main finding of **Study IV** was that silicone oil significantly decreased the capacitance increase produced by coating of albumin or fHb solutions on the surface of the measuring chamber of the studied capacitance-based AU, which already had been validated to continuously measure UO in **Study I** and **II** (30, 106, 107). However, before performing **Study I** and **II** we had clinically experienced that biofilm coating of the AU by fHb and albumin in urine from patients undergoing cardiac surgery with CPB affected AU measurements. This initiated the manufacturer to include a water-dissolvable capsule containing silicone oil in its antechamber before we conducted **Study I** and **II**. However, we still wanted to investigate the impact of fHb and albumin on the new version of the AU with an enclosed silicone oil (**Study IV**).

In **Study IV**, both the albumin (3 g/L) and the fHb solution significantly amplified the capacitance measurement in the AU. Silicone oil released from the antechamber of the AU significantly decreased those increases in capacitance during the 23-hour study period, by at least partially prevent biofilm formation in the measuring chamber. To our knowledge, this is the first investigation indicating at least partial protection of an albumin and fHb biofilm, respectively, on plastic surfaces by use of silicone oil.

In a large clinical study involving 1200 patients undergoing coronary surgery (52), 80% of patients had early postoperative albumin levels in urine of 0.05 g/L or lower. Thus, our studied albumin solution 3 g/L had a significantly higher concentration than the concentration of albumin in urine found postoperatively in at least 80% of patients undergoing conventional cardiac surgery, implying that the beneficial effect of silicone oil should apply to the vast majority of conventional postoperative cardiac patients.

## 5.3 CLINICAL IMPLICATIONS

### 5.3.1 Study I and II

When using an MU for measuring UO, the staff is required to read and empty the MU manually at specific time intervals. This is unfortunately frequently impractical or impossible due to shortage of nursing staff. However, if this is possible, **Study I** and **Study II** show that the MU measures hourly UO adequately and similarly to the AU. Yet, irrespective of staff number, the AU may give the staff the opportunity to perform other responsibilities. Likewise, using the AU in normal wards may allow hourly UO measurements in all patients with a urinary catheter, and not restrict measurements to

infrequent daily episodes. This may, together with laboratory kidney parameters, enable a faster and more accurate diagnosis using current AKI criteria. Theoretically, the higher measurement resolution that may be obtained with the AU should benefit early detection of an upcoming AKI. The AU may also potentially decrease the risk of retrograde urinary tract contamination, as it will minimize the nursing staff´s direct contact with the system and avoid the requirement for the nursing staff to bend down with possible contamination of clothes or hands from the floor or bed while reading the scale of the MU. Another disadvantage of the MU may ensue during high UO. If the UO tops 500 mL/hour, the MU will not include the complete volume in the measuring chambers, and the volume above 500 mL with be drained directly into the urine bag. Thus, this surplus volume can only roughly be judged hourly if the urine bag is emptied or measured with the inexact scale of the urine bag. However, in **Study I** we circumvented this potential problem by emptying the urinary bag hourly.

We are not aware of the costs of the AU but according to the manufacturer the price setting will probably be close to that of the MU. When the exact cost is known, one could conduct a cost-effectiveness study. The clinician has to evaluate the potential added value of the AU, when taking into consideration its potential advantages including time saving in low staffed wards and possibility of automatic data transfer avoiding human errors in data entries.

In addition to the silicone oil of medium viscosity applied in the measuring chamber of the AU, a supplementary low viscosity silicone oil that coats the proximal end of the tubing towards the AU could potentially have additional advantages. Moreover, reiterating silicone oil would recoat the surface.

### 5.3.2 Study III

The findings in **Study III** may support the development of novel surfaces that repel biofilm formation. Upcoming studies need to investigate the duration of silicone oil on biofilm formation and the potential clinical benefit in this respect of the new AU. Silicone oil, in contrast to silicone, has so far not been used in the urinary catheters, but we believe this may be beneficial. However, this has to be evaluated in a follow-up trial and the effect will probably depend on the plastic used in the urinary catheter, the amount of delivered silicone oil and the frequency of oil replenishment.

### 5.3.3 Study IV

The clinical importance of **Study IV** is that supplementation of silicone oil in the capacitance-based AU allows for prolonged measurements of trustworthy UO until its throwaway part has to be substituted. This would be of value in patients with fHb and/or albumin in urine, which often is the case for example in patients who have undergone cardiac surgery with CPB, in patients with diabetes mellitus, and in patients with renal dysfunction. Indeed, we have not seen failed AU measurements early after cardiac surgery since silicone oil was included in AU.

## 5.4 LIMITATIONS

### 5.4.1 Study I

First, we did not randomize the patients. Instead, we allocated patients to the urinometer that was currently in use. Thus, we performed **Study I** during two stages, whereby we first investigated the MU, followed by an evaluation of the AU. Second, there was a difference in the mean of the hourly urine cylinder reference measurements between the MU and the AU groups whereby a higher mean was found in the MU group. This may have been a potential bias. However, we found similar results in both ranges compared to the overall result when we performed subgroup analyses that demarcated each urinometer group at hourly UO values of 100 mL (**Table 4**). Furthermore, we omitted measurements if the nursing staff had unintentionally placed the AU or the MU erroneously. This occurred almost solely when the nursing staff mobilized the patient from the bed to a chair. Additional education should help to prevent this problem with both the AU and the MU, although this drawback is likely not distinguished as clearly with the MU as with the AU. Third, one could argue that only 408 comparisons were made in 36 post cardiac surgery patients (less than 12 hours per patient). This seems to be short compared with the ICU length of stay for cardiac surgery patients. However, a similar study with a droplet-based AU (23) included 453 measurements and was the base for our power calculation. Our measurements were for practical reasons not conducted during nighttime, as a laboratory technician was needed to conduct the reference measurements.

### 5.4.2 Study II

First, as in **Study I,** we did not randomize patients in **Study II** and instead allocated the patients to either the MU or the AU. Second, one might see the variation of number of measurements per patient as a selection bias. Third, there were fewer measurements with

the MU than with the AU (83 vs. 127), and the number of patients in each group, though similar, were few, with six patients in the MU group and eight patients in the AU group. **Study II** should rather have included more patients and several centers. Fourth, more diverse patients and specifically patients with manifest oliguria would have been preferable. However, assuming a cutoff for oliguria at <0.5mL/kg/h, 9% and 7% of the measurement in the AU and MU group, respectively, were oliguric. Anyway, we consider performing a larger study taking these points into consideration. Fifth, one may ask why there were wide fluctuations with both the AU and the MU, specifically in the nine cases with biases above 10 mL. In fact, there were five instances with biases of more than 10 mL, three with the AU and two with the MU in four patients. Regarding the AU, the measurements surrounding the measurements with high bias (measurements one hour before and one hour after) were normal (<5mL bias). We can only speculate about the reasons. Of course, there may have been inaccurate measurements involved with any of the measuring methods including the cylinder.

Lastly, as the AU adapts the measurement of urine to the position of the device and sets off an alarm should the position interfere with measurement, it is conceivable this is a potential issue given the often very frequent movement of the pediatric ICU patient. However, before the start of **Study II**, we had a rather large experience of using the AU in the adult ICU, and the alarm set off for position interference with measurement very infrequently, in fact only when the patients were transported for external evaluations, like CT-scans. Neither in pediatric nor in adult patients did we experience alarms due to movements by the patient while still in the bed.

### 5.4.3 Study III

First, we did not measure the exact width of the silicone oil coating and how the silicone oil was dispersed in the wells. One could presume that there should be more oil in the bottom of the well than on the walls, as oil is likely to slide down. Indeed, we detected more biofilm on the walls compared with the bottom of the wells. Conversely, this may as well be explained by how biofilm is formed (108). Anyway, we assumed that the quantity of oil on the walls was similar between wells. Still, further studies should quantify the spreading of silicone oil in the measuring chamber of the AU as well as if oil thickness varies over time. Second, we do not know for how long the influence of silicone oil lasts. In **Study III**, we recorded measurements for 72 hours, because most patients using the AU will not stay longer in the ICU after cardiac procedures. Furthermore, we found that the biofilm formation reached a plateau phase after 72 hours.

### 5.4.4 Study IV

First, in **Study IV** we applied Ringer's acetate as a surrogate for normal urine. Admittedly, we may have utilized more complex formulas for artificial urine (109), but we chose to use Ringer's acetate as it is cheap and clinically accessible and mixes well with albumin and fHb. Moreover, it is both difficult and expensive to make large quantities of sterile artificial urine. There were several arguments against using humane urine. We would have needed approximately 80 L of human urine, which also may have a large interpersonal variation in electrolyte composition and pH, and it is complicated to keep such large volumes sterile. Indeed, other investigators have applied similar solutions as we did to replace human urine, e.g. Rasmussen et al. studied ascending infections using 0.9% saline (110). Second, our experiments were restricted to 24 hours, and with a longer follow-up time we might have seen significant effects with both the 0.3 and 1.0 g/L albumin solution. Third, the concentration of fHb varied somewhat between each run. However, the mean difference in concentration in fHb was very similar between both groups and should accordingly only marginally have had an impact on the results (0.0113 g/L with silicone oil and 0.0125 g/L without silicone oil). Interestingly, fHb concentrations in urine in patients with discolored urine in the first hours after cardiac surgery with CPB, typically fluctuate between 0.1 to 5 g/L in our cardiothoracic ICU, after which it regularly drops. Thus, in our study we used a fHb concentration that was approximately 10% of the real values at most. Conversely, we have not experienced malfunctioning measurements in our clinical routine since we started to use the new AU Sippi® device with an enclosed water-soluble capsule with silicone oil. Fourth, **Study IV** was neither randomized nor blinded, but as data measurements were automatic, this should not have influenced the results.

# 6 CONCLUSIONS

The specific conclusions were:

- This study compared the performance of a new capacitance-based AU with that of an MU in adult patients in a cardiothoracic ICU. The AU was non-inferior to the MU regarding precision, and significantly better than the MU in terms of bias, temporal deviation and staff opinion.

- When the modified capacitance-based AU was compared with a standard MU in a pedatric intensive care unit for children weighing ≤10 kg, the urinometers were comparable regarding bias of measurements. Staff consistently rated the modified AU significantly higher than the MU in terms of user friendliness, time-consumption and duration of contact with the urine bag.

- A silicone oil-coated polypropylene plastic surface, as used in an AU, significantly decreased early biofilm formation by pathogenic bacteria, including ESBL-producing and multidrug resistant strains, as well as *C. albicans.* Both low and medium viscosity silicone oils significantly reduced early biofilm formation to a similar extent.

- Curli fimbriae appeared to be the tentative silicone oil target and addition of silicone oil led to a 50% reduction in biofilm formation in curli-positive strains. No additional reduction in biofilm formation could be detected when adding silicone oil to curli deficient strains.

- Albumin or free hemoglobin coating of the capacitance measurement membrane of the AU decreased the capacitance measurement capability of the AU, and this could significantly be attenuated by addition of silicone oil to the measuring chamber of the device.

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