I.N. Brouwer

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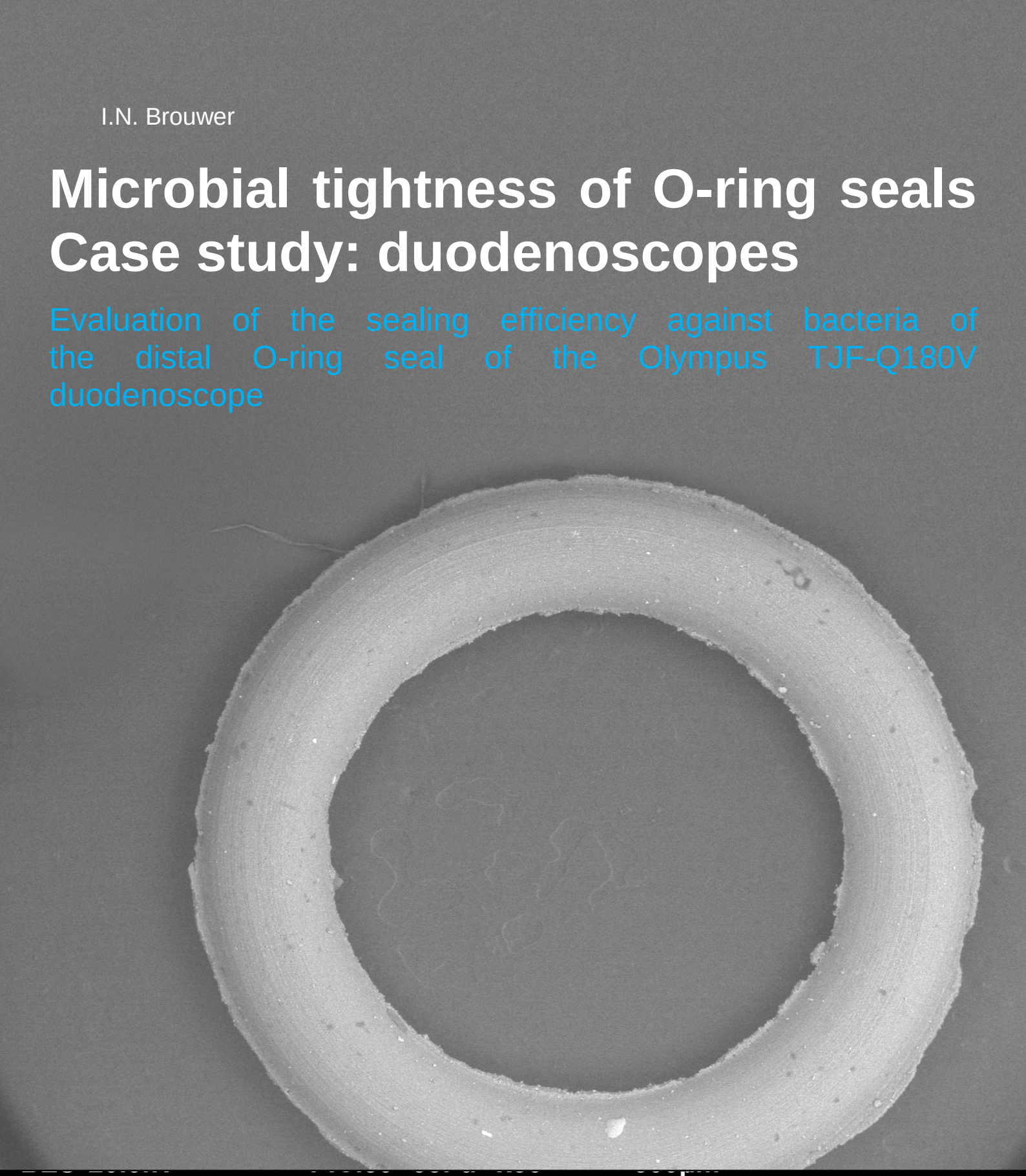

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Microbial tightness of O-ring seals
Case study: duodenoscopes

Evaluation of the sealing efficiency against bacteria of
the distal O-ring seal
of the Olympus TJF-Q180V duodenoscope

By

I.N. Brouwer

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This thesis is confidential and cannot be made public until the 1st of October, 2018

The picture on the cover page is made by Dr. ir. Iulian Apachitei

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Main conclusions of this thesis

- O-ring sealing constructions that have efficient sealing against air do not necessarily have efficient sealing against micro-organisms, even in a static construction.
- Dynamic rotational loading of the axle of an airtight O-ring sealing construction can have a significant effect on bacterial penetration through airtight seals.
- The leakage test performed in clinical settings might ineffectively test the sealing efficacy of the O-ring seal in the elevator construction of distally sealed duodenoscopes.

Preface

This thesis is the result of a ten-month graduation project at the Medical Microbiology (MMB) department section Infection Prevention of University Medical Center Groningen (UMCG) and the Technical University (TU) Delft, succeeding to the literature thesis 'Is the design of new model duodenoscopes truly impeding reprocessing?'. The project was set up to fulfill the final requirements for the Master 'Biomedical Engineering' track 'Medical Instruments & Medical Safety'.

With my interest in the invisible microbial world and motivation to improve current medical procedures or instruments, I was longed to find a graduation project on optimizing cleaning, disinfecting or sterilizing medical instruments to prevent infections. This wish was perfectly compatible with the department Biomedical Engineering at the TU Delft; the department had experience investigating duodenoscopes and had the ambition to study bacterial leakage in medical devices. The Infection Prevention of the UMCG offered a graduation internship at their department to clinically support the study.

On first glance this thesis is a descriptive study; the cause of duodenoscope-related infections is tried to be found by evaluating leakage of the O-ring seal in its distal tip. However, looking more closely to the discussion chapter of this thesis, the reader will discover that this is a predictive research too. In the field of minimally invasive surgery, O-ring seals are often applied, and likely will be even more frequently applied as a result of the increasing engineering complexity in this field.

I believe that I learned a lot during this project in several aspects; mechanical design, experimental methods and project management. I have covered up with overdue practical experience with mechanical design resulting from my more biomedical science-oriented bachelor. Besides, my communication skills are developed further, since required information management was not easy due to the political sensitiveness of the subject infection safety of duodenoscopes. Moreover, I gained more self-confidence with self-reliant decision making; since studies on bacterial penetration through seals are a quite undetermined field, I had to make many decisions about the study approach.

Finally, I would like to mention that I am very satisfied that this graduation project contributed to getting a job in the field of infection safety for medical instruments in the UMCG.

I hope this study inspires others to study microbiological safety of medical instruments; even though effects seem invisible, it is an important aspect of keeping patients healthy. In any case, thank you for taking the time to have a look at my thesis.

Kind regards,

I.N. Brouwer

Groningen, February 2017

Construction of the thesis

In the first chapter, the background information is given of both O-ring sealing and duodenoscopes required to round up this chapter with comprehensible study goals of this thesis. The main text of the thesis consists out of three parts, *Part A*, *B* and *C*, all evaluating another aspect of the tightness of O-ring seals in medical devices. Part A elaborates on O-ring seals used in medical instruments in general. Then, *Part B* focuses on the air tightness of the O-ring seal in pre-recall Olympus TJF-Q180V duodenoscopes, and *Part C* describes two separate investigations on the bacterial tightness in pre-recall Olympus TJF-Q180V duodenoscopes. All findings are briefly discussed at the end of each Chapter, which are on their turn all combined in the final discussion in *Chapter 8*.

The main study of this thesis is described in the last chapter in *Part C*, *Chapter 7*, and therefor contains the most relevant results. Based on the contents of this Chapter, an article is written intended to be submitted to the Journal *Nature Biomedical Engineering*. This article is included at the very beginning of this thesis.

Evaluating bacterial tightness of seals in a systematic manner using duodenoscopes as case study

Abstract

Sealing constructions are frequently used in reusable instruments to prevent the loss of a fluid or gas in a construction by obstructing the flow of fluids or gasses through the glands in the interface of two or more separate parts, and may be in direct contact with the patient's body fluids. With the increase of infections associated with multi-drug resistant bacteria, information about the microbial tightness of seals becomes increasingly more important.

The bacterial tightness of the O-ring sealing construction was evaluated by a systematic investigation studying bacterial tightness of airtight reproductions (at 240 mbar) of the distal sealing mechanism in of Olympus TJF-Q180V duodenoscopes. During test runs, product- and usage variables -expected to influence microbial leakage- were closely controlled.

Here, we show that airtight O-ring seals can leak bacteria, even in static conditions, and moreover, rotation of the axle is a significant microbe-influencing variable. With regards to infections prevention, medical designers, manufacturers and safety controllers should have knowledge of this insight. More systematic research in this manner can add valuable information.

Keywords; seals, seal tightness, bacterial tightness, microleakage, microbial leakage, bacterial leakage, duodenoscopes, ERCP

Introduction

Seals prevent the loss of a fluid or gas in a construction, by obstructing the flow of fluids or gasses through glands in the interface of two or more parts. O-rings are most frequently used since they are easy to design and manufacture, cheap and an effective. [1]–[4] O-rings are doughnut-shaped rings molded from elastomers, thermoplastic materials or metal and available in a wide range of sizes. The sealing construction consists of an O-ring in a gland in its housing; the O-ring is compressed between the gland and its counter surface, and therewith sealing is provided by the material's elasticity (Figure 1).

In medical devices seals are used to prevent fluid flow -for example of patient material or reprocessing disinfectants - to invade into its internal parts –for example, to protect its electrical components-. Based on seal-patient-interaction seals in medical devices may be divided into two groups; those in direct contact and in indirect contact with patient material. Seals in direct contact with patient material prevent the loss of the patients' fluids and -gasses (i.e. blood, airway gasses, intestinal fluid and urine) in a construction: the dirty side of the seal can contact patient material whole the other side of the seal stays clean. If the one side of an O-ring is freely accessible for patient material, leakage of micro-organisms to the other side of the O-ring should ideally be prevented.

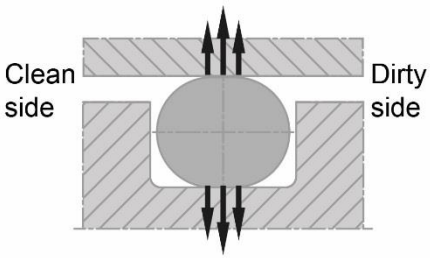

Figure 1. Cross section of an O-ring in direct contact with patient material. The O-ring separates the dirty and clean side.

Theoretically, micro-organisms will not leak through seals if there is no leakage of fluids and gasses since micro-organisms' sizes are larger than singular molecules; for the

leakage of bacterial marginal gaps are needed of at least 0.5µm-1.0µm. [5] However, it is important to keep in mind that bacteria are living organisms; they might actively migrate and promote leakage, and moreover one leaked bacteria could duplicate, while one molecule of air of fluid just stays one molecule. Besides, even for well-performing seals, gasses or fluids may leak in small amounts; molecules can pass along non-contact areas -only visible at high magnification- at the interface of the O-ring and its housing.[6] Therefore, it could be a crucial misconception that air- and fluid leakage and microbial leakage are inherent; absence of visible air leakage does not necessarily have to exclude leakage of microorganisms. Knowledge of the microbial sealing behavior of seals in medical devices is important with regards to prevention of infections.

A duodenoscope is a medical device with a seal in direct contact with patient material and disinfectants. The sudden appearance of MDRO infections associated with duodenoscopes (about 80 each year) is frequently discussed, and the parallel introduction of the O-ring in the distal tip has been put forward as the cause.[7]–[11] Duodenoscopes are sold by three different manufacturers of which Olympus has a market share of 85%.Therefore, the O-ring seal in Olympus TJF-Q180V duodenoscopes is used as case study to study microbial tightness of seals.[12], [13]

We describe the design and findings of a systematic study investigating to the bacterial tightness of airtight sealing constructions. We demonstrate that an airtight seal construction according to the standards does not necessarily have to be an airtight seal. Using faithful reproductions of Olympus TJF Q180V duodenoscopes, the airtightness at 240mbar and bacterial tightness to Klebsiella Pneumonia is evaluated in standardized test runs with static conditions, pressurization of the dirty side and dynamics of the axle including axial play. This indicates that bacterial leakage may occur in airtight O-ring sealing constructions and that dynamically rotational loading of the axle has a significant influence on the frequency of leakage.

Sealing constructions in medical devices

Medical grade O-rings are widely sold by several manufacturers for many types of medical instruments. According to manufacturer themselves they are sold for medical appliances, dialyzers, medical pumps, intravenous components, feeding devices, implant materials, non-implantable instruments, autoclaves and medical- and diagnostics instruments. [14],[15] Even though this claim, an overview of their application in medical products is hard to make: O-rings are installed inside a housing and are thereby, most often, ‘hidden’ into the construction.

If O-ring seals can leak bacteria through the seal, it is likely that adverse events (AE’s) related to O-ring seals have been reported. However, these reported adverse events are probably an incomplete reflection; most likely it shows only ‘a tip of the iceberg’ since exogenous cross-infection infections can be unrecognizable or remain underreported or unnoticed. [16]–[18]

A systematic search was done in the databases Scopus and Pubmed to the reported infection safety adverse events related to O-ring seals to obtain a reflection of the current state of knowledge of infection safety of O-rings applied in medical instruments. Two instruments were reported with O-ring-related AE’s (Figure 2). Firstly, several studies have reported dialyzers with O-rings and its housing contaminated with bacteria after

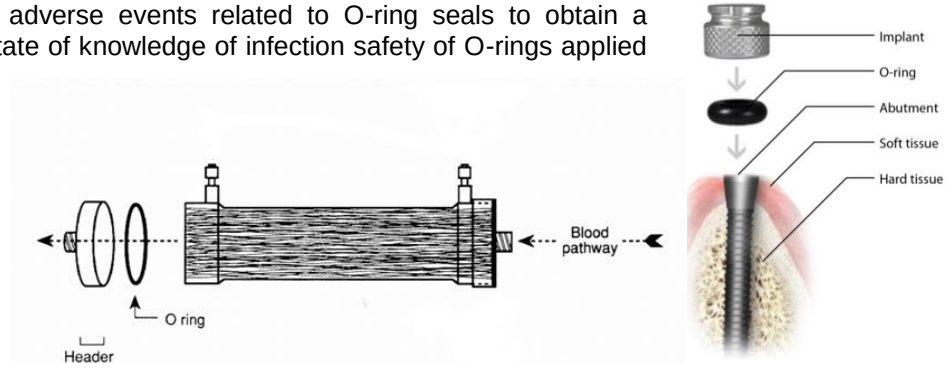

Figure 2. Abstract drawing of medical devices associated with O-ring-related AE’s. Left: dental implant. Right: dialyzer.

reprocessing. To prevent leakage between the blood compartments and its headers, an O-ring is functioning as a gasket: to O-ring is installed in an axial groove in the header and provides sealing by compression between the header and the compartment. [19], [20], [21], [22] Secondly, bacterial leakage through O-rings was reported in a systematic investigation in dental implants; the O-ring group had 20 times more leakage bacterial leakage than the gel-sealed group. [23] Most dental implants have two pieces: the abutment, functioning as the dental root, and the tooth implant functioning as the new teeth, of which the interface is sealed to prevent infections.

Sealing construction in ERCP-endoscopes

Duodenoscopes are a special type flexible endoscope adapted to perform endoscopic cholangiopancreatography (ERCP) procedures in the biliary tree. To enable physicians to guide surgical instruments in the major duodenal papilla, a special feature is made at the duodenoscopes distal tip: a forceps elevator adjustable by a knob on the control unit (Figure 3). All the three manufacturers have duodenoscopes in two models: an older model, and a new model -updated in 2009- with some design adaptions. From the design point of view, the channel which guides the elevator wire -connecting the forceps elevator with the control knob- is now sealed instead of freely accessible. Instead of the older models, updated model duodenoscopes have an O-ring seal at the axle of the elevator lever that should fully obstruct the elevator wire channel, and thereby cancels out the need for reprocessing of this channel (Figure 3).

One investigation of a duodenoscope have been found brownish debris at the clean side of the O-ring.[13] If the O-ring leaks microorganisms to the clean side of the construction during its life cycle, the clean side of the axle and lever recess could turn into a micro-reservoir for bacteria.[23]–[26],[27] Since migration of microorganisms back to the elevator recess cannot be excluded when contamination of the clean side can occur, concealed microorganisms in the contaminated clean side of an O-ring construction can consequently be discharged into the patient during an ERCP procedure. Entrapped bacteria are unlikely to be killed during the reprocessing.

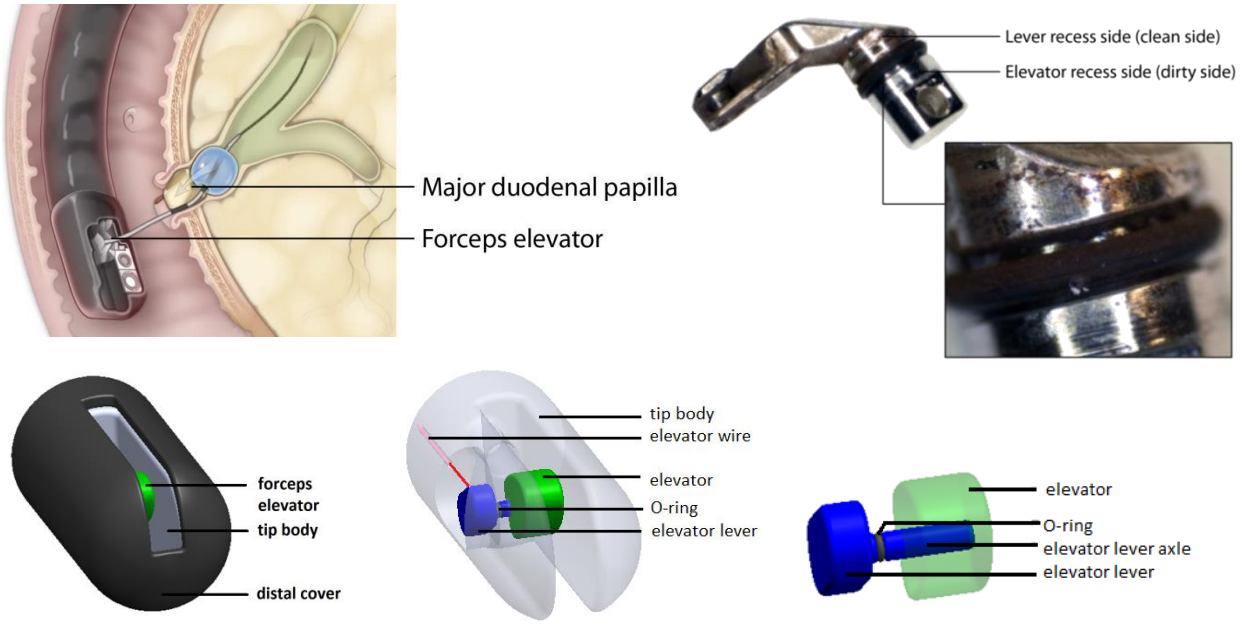

Figure 3. The forceps elevator at the distal tip of duodenoscopes. Top-left: The elevator is used to manipulates accessories into the papilla. Top-right: brownish debris has been detected on the clean side of the O-ring. Bottom: the distal tip has a fixated distal cover and a forceps elevator. The forceps elevator is connected with an elevator lever with an O-ring shielding the elevator wire channel.

Reproducing a sealing construction to an experimental setup

A closely controlled investigation could give detailed insights info microbial sealing behavior of seals. We made an inventory of the variables that may have influence on microbial leakage with a distinction between factors introduced by varieties in the product and by varieties introduced by use (Table 1). With such this list, a systematic investigation can be designed; variables of interest must be varied while the others need to be kept constant.

The experiment setup for the systematic investigation must include a seal separating the clean and non-clean side (Figure 4). The seal's dimensions should be manufactured according to the ISO-standards holding close tolerances. As a reference for the performance of the seal, the air- or fluid tightness should be measured. Ideally, the experiment setup is replicated, to reduce the effect of manufacturing deviations

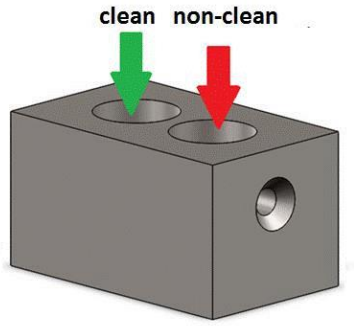

Figure 4. Experiment setup separating a clean and non-clean side

Table 1. List with all variables that could possibly influence microbial leakage.

Product variables	Usage variables
• Bearing length • Radial clearance • Effective compression of the installed O-ring • Hardness of the O-ring • Material of the housing • Material of the O-ring • Cross-section of the O-ring • Stretch of the installed O-ring • Surface finish of the housing at the seal-contact-surface • Surface finish of the O-ring	• Angel of rotation of the axle • Angular displacement of the axle • Axial displacement of the axle • Condition of the O-ring (aging) • Density and viscosity of the medium at the dirty side • Frequency of rotation of the axle • Pressure at the dirty side • Speed of rotation of the axle • Type of microorganism(s) • Environmental temperature

Evaluating bacterial tightness of a sealing construction

After systematically controlled test runs simulating usage, the bacterial tightness can be evaluated; therefore bacterial penetration from the dirty to the clean side must be measured. An indication of bacteria presence is done by sampling followed by culturing; both can be done by various methods. Based on important requirements for the sampling method, as listed in the table, the most suitable sampling method seems to be BHI broth immersed at the clean side after a test run (Table 2). Also for the culturing method, important requirements are listed in the table. The most suitable sampling method seems to counting the number of colony forming units (CFU) of a sample (Table 2). Bacterial penetration studies should interpret their data rather qualitative than quantitative, due to the difficulty in controlling the bacterial population.[5] It is feasible to use one type of (identified) bacteria strain since this eases the evaluation. To preclude false positive results, the experimental setup must exclude environmental microbiological contamination.

Systematic evaluation of the bacterial tightness of reproduced duodenoscope sealing constructions

Reproductions of the distal O-ring seal in the elevator mechanism of Olympus TJF-Q180V duodenoscopes were used to evaluate bacterial leakage in a systematic investigation simulating use. The experiment set-up was a triple parallel reproduction of the construction in Olympus TJF-Q180V duodenoscopes (Figure 5). The axles were assembled using an aligner.

Table 2. Weighting methods on important requirements for a systematic investigation. Top: Sampling method. Bottom: Culturing method.

Sampling method		**Swab the clean side after test runs**		**BHI broth immersed into at the clean side during test runs**		**BHI broth immersed at the clean side after test runs**	
Requirement	Weight	Score	Weighted score	Score	Weighted score	Score	Weighted score
High sampling-density of surfaces	3	1	3	3	9	2	6
Possibility for spatial leakage measurement	1	2	2	3	3	1	1
Sampling should not affect results	4	2	8	1	4	3	12
Total scoring for requirement fulfillment			**13**	**16**		**19**	

Culturing method		**ATP level**		**#CFU in broth**		**Turbidity of broth**	
Requirement	Weight	Score	Weighted score	Score	Weighted score	Score	Weighted score
Determination of bacteria possible (i.e. identify environmental contamination)	4	1	4	3	12	2	8
Quantification of results possible	3	3	9	3	9	1	3
Total scoring for requirement fulfillment			**13**	**21**		**11**	

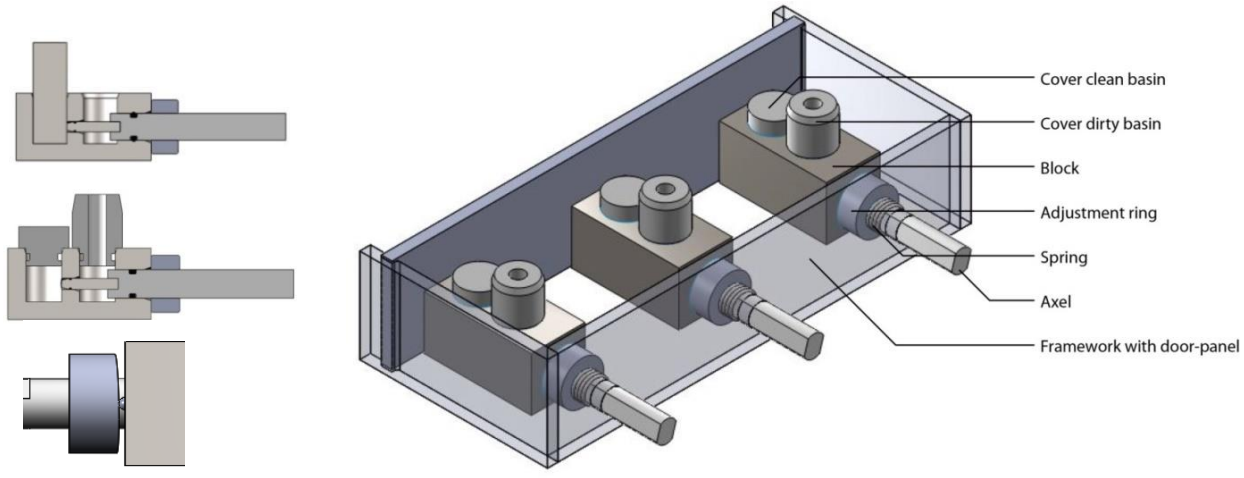

Figure 5. Experiment construction. Left-top: an aligner is used to align the axles. Left-middle: covers are used to prevent cross-contamination. Left-bottom: angles adjustment is used to induce translational motion of a rotating axle. Right: complete experiment construction, replications in its support construction.

In clinical settings, the distal O-ring seal has various factors that possibly can influence the sealing efficiency against micro-organisms. The reproductions were inoculated with a full growth suspension of Klebsiella Pneumonia –type of bacteria often associated with duodenoscope-related infections- and submitted to usage simulation in test runs for 1±0.01hour – the maximum duration of an ERCP procedure- in a standardized manner. The usage variables chosen as an independent variable for this investigation are listed below. Other products- and usage variables were kept constant to a value optimal for the sealing performance according to the standards.

- **The pressure at the dirty side was set at 16 mbar.** The pressure at the dirty side of the seal in the distal tip of duodenoscopes could be created inside the patient's body or during reprocessing resulting from the flowing cleaning and disinfection fluids. Pressure resulting from reprocessing is not defined, however, is expected to be negligible, since the seal is sheltered from the flow. The intra-abdominal pressure during ERCP is reported in bovine animals to be maximal 16 mbar.[28] The pressure is created by a bacteria fluid column filled to 16.7 cm.

- **The frequency of rotation of the axle was set at 30 times 90°.** The frequency of manipulation of the forceps elevator was maximally estimated on 30 times during ERCP (accessories instrument exchanges is maximally 6 [29] for each exchange the number of manipulations of the forceps elevator was estimated at 5 times). The axles were manually rotated for 90° each minute.

- **Axial displacement was set at 0.375mm.** The maximal axial play was estimated by observing the forceps elevator of an Olympus TJF-Q160V duodenoscope. The axial displacement was created by a 3-mm bullet combined with an angled bearing surface (Figure 5).

Before each test run, the airtightness of the reproductions was tested using a manual adapted sphygmomanometer, pressurizing the clean side to 240 mbar (frequently used the pressure of the leakage test in flexible endoscopes); a dropping pressure the seal was indicated a non-airtight and a constant pressure indicated an airtight seal. During the test runs measures were taken to prevent environmental contamination, also including negative controls to check for possibly resulting in false positive results. After each test run, the clean side was sampled with indirect immersion, cultured and evaluated by counting the CFU and identifying with Maldi-Tof MS. CFU's from bacteria strains not being K. Pneumonia –and therefore environmental contamination- were excluded from the results.

All cultures originating from the negative controls showed no growth. Bacterial penetration was found in all groups (Figure 6). The levels in each group were compared for the presence of a statistically significant difference in the equality of proportions using two-tailed Chi-Square tests. Airtight seals submitted to gauge pressure at the dirty side were equally likely to have bacterial penetration (9%) seals with no applied gauge pressure (10%) ($\chi^2(1, N = 21) = 0.00502$, $p = .05$). On the contrary, airtight seals with dynamical axles were significantly more likely to have bacterial penetration (60%) than in reproductions with passive axles (10%) ($\chi^2(1, N = 20) = 5.50$, $p = .05$). Also, airtight seals with dynamically-translational axles were significantly more likely to have bacterial penetration (67%) than reproductions with passive axles (10%) ($\chi^2(1, N = 19) = 6.54$, $p = .05$). However, airtight seals with dynamically-translating axles were equally likely to have microbial leakage (67%) as seals of reproductions with dynamically, non-translating axles (60%) ($\chi^2(1, N = 19) = 0.0905$, $p = .05$).

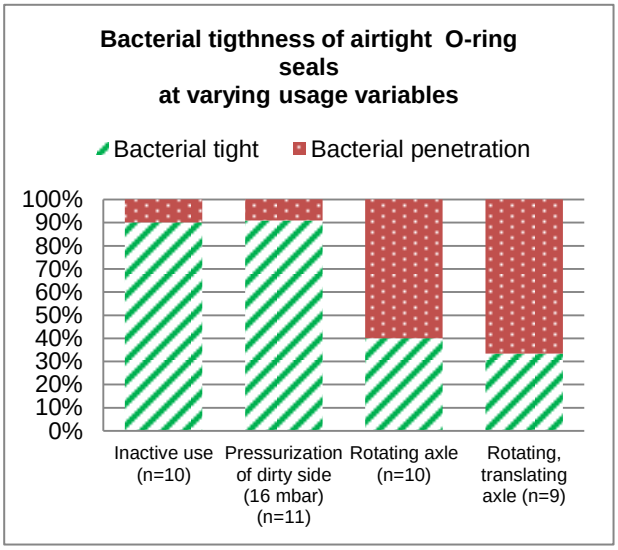

Figure 6. Results of the systematic investigation of bacterial tightness of reporductions of the distal sealing mechanism of duododenoscopes

Discussion

The results suggest that in the distal O-ring seal in the elevator mechanism duodenoscopes is likely to leak micro-bacteria to the clean side and back to the dirty side; the faithful reproductions leak micro-organisms in passive variables, and bacterial leakage significantly increases when the forceps elevator is used during the ERCP procedure. The best-case sealing constructions were used for the test runs with the worst-case usage variables. Therefore, the results of this controlled investigation are an overestimation of the bacterial tightness during maximum use intensity. It must be emphasized that the controlled experiment is only an investigation approaching the sealing efficiency against micro-organisms of the distal seal in the Olympus TJF-Q180V in an ERCP procedure. Even dough they are a highly similar reproduction, some reproduction dimensions slightly deviate from the original, besides the surface roughness and seal's material type were not taken into account into the setup. To be able to make conclusions about the distal sealing mechanism in duodenosocpes, the protocol of this experiment should be applied on the seals in actual duodenoscopes.

Using duodenoscopes as a case study, the systematic investigation proves that an airtight seal in passive conditions can leak microorganisms and that certain variables can significantly increase the presence of this leakage during instrument use. If an O-ring seal is leaking bacteria, then a logical consequence is that the small voids in the O-ring housing and after the O-ring housing also may harbor bacteria. Reuse of instruments with O-rings in direct contact with patient material may thereby pose a risk to infection safety for patients since the microorganisms trapped in the O-ring sealing will be hard to effectively kill during reprocessing. Microbe leaking O-rings may especially be a problem for medical instruments that are reprocessed with chemical disinfection, since with this method reduction of vivid microbes is obtained, unlike of thermal disinfection and sterilization, by contact between microbes on the instruments' surfaces and disinfectants.

This systematic evaluation of bacterial tightness of seals can be a start in a series of studies creating insight in the microbial sealing behavior of seals in medical devices. This would add valuable information to give researchers and developers of medical instruments. Further studies could replicate the test runs to a higher number than the current systematic investigation: for the statistical test applied a minimum of 5 results per level for each group would have been preferable, hover this is not applicable for the bacterial penetration for the groups 'usage variables zero' and '16 mbar on dirty side'. Moreover, it would be interesting to also evaluate bacterial tightness for more factors that may influence leakage of microorganisms, for example angular displacement is expected to influence the bacterial sealing efficacy since it alters the effective compression along the sealing surface or the bacteria type studies since bacteria could actively migrate across the seal, for example, if having flagella. Also, it may be interesting to evaluate various types of sealing constructions –for example, Bodok seals, basic lids, inflatable seals, ferrofluidic gels, kits, and diaphragm seals - in various sizes, all designed and manufactured in accordance with the ISO standards. For O-rings, the standards listed in ISO3601-2 could be used. [30]

Methods

Systematic literature search

Databases examined are *Scopus* and *PubMed*. The search line used is a combination of the keywords '*(o-ring OR gasket) and (infect* OR outbreak OR contaminated*) and (bacter* OR micro*)'.* Articles are included with the following criteria;

- The reported problem with the described O-ring must be associated with infection prevention;
- The related O-ring seal must be used in a reusable medical instrument.

The search resulted in Scopus 103 results and Pubmed 27 results, of which 5 passed the selection criteria. These 5 relevant results reported 2 unique reusable medical instruments with AE's of O-rings infection safety. These reusable medical instruments are dialyzers and dental implants

Experiment setup

The set-up consisted of three aspects: the experiment construction, bacteria suspension, and leakage tester. The construction was built from of several parts; a trifold parallel reproduction, installation assistance parts, support assistance parts, parts for motion simulation, parts for pressurization simulation. The trifold parallel mockup construction were made from 3 stainless steel blocks, 3 stainless steel axles, and silicone O-rings (Apple Rubber Products BV, compound designation: SL) and were designed and manufactured in accordance with ISO 3601-2 with the dimensions the elevator mechanism as measured in the Olympus TJF-Q180V (*Figure 7*, Table 3 and Table 4). Clean side covers and dirty side pump adapters were made to prevent environmental contamination. The installation assistance parts were one stainless steel aligner and three adjustment rings. To facilitate translational motion of the axles, a sliding system was used; three stainless steel angled adjustment rings and 3 stainless steel 3mm bullet. The support assistance parts were a stainless steel framework with a sliding door panel and three stainless steel springs. Fluid columns (25 mL Greiner CELLSTAR® serological pipette) were used to simulate gauge pressure on the dirty side of the elevator lever system; with a full growth media density of 0.96*103 kg/m3 the fluid was filled to 16.7 cm to have 16 mbar gauge pressure.

The bacteria suspension used was fresh full-growth BHI broth of a fully antibiotic-sensitive K. Pneumonia strain (ATCC 13883) stored in the fridge at 9°C for maximally 3 days. The leakage tester was an adapted manual sphygmomanometer (0-400 mmHg Heine Gamma XL); using a T-connection 6x6x6mm the dial is connected to the handcuff and to a 6x13 mm straight connector connected to a 13mm PCV outlet tube fitting to the dirty side pump connector.

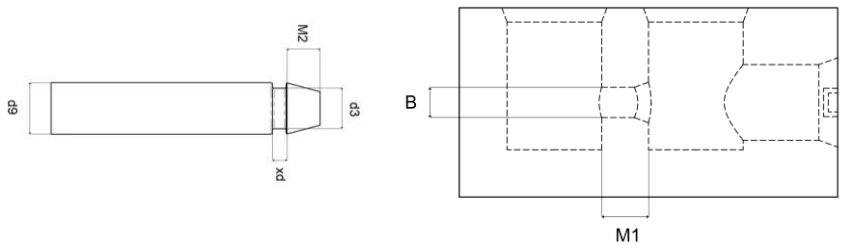

Figure 7. Formulae used to calculate dimensions not directly measurable in an O-ring sealing

Table 3. Construction drawing of the axle and block of the experiment construction.

Dimension	Symbol	Formula [31]
Radial clearance	g_{min}	$= (d4_{min} - d9_{max})/2$
	g_{max}	$= (d4_{max} - d9_{min})/2$
Radial housing depth	t_{min}	$=(d4_{min} - d3_{max})/2$
	t_{max}	$=(d4_{max} - d3_{min})/2$
Diametrical stretch inside diameter O-ring installed (mm)	s_{min}	$=d3_{min} - d1_{max}$
	s_{max}	$=d3_{max} - d1_{min}$
Percentage of inside diameter stretch	S_{min}	$=(s_{min}/d1_{max})*100[\%]$
	S_{max}	$=(s_{max}/d1_{min})*100[\%]$
Percentage of O-ring cross-sectional reduction resulting from diametric stretch	R_{min}	$= 0{,}01 + 1{,}06(S_{min}) - 0{,}1 (S_{min})^2$
	R_{max}	$= 0{,}01 + 1{,}06(S_{max}) - 0{,}1 (S_{max})^2$
Cross section installed O-ring (mm)	$d2^*_{min}$	$=d2_{min} - d2_{min}(R_{max}/100)$
	$d2^*_{max}$	$=d2_{max} - d2_{max}(R_{min}/100)$
Percentage of effective O-ring cross-section compression	C_{min}	$=(d2^*_{min} - t_{max})/d2^*_{min}*100[\%]$
	C_{max}	$=(d2^*_{max} - t_{min})/d2^*_{max}*100[\%]$
Bearing length	M_{min}	$=M1_{min} - z1_{max} - z2_{max} - dx_{max}$
	M_{max}	$=M1_{max} - z1_{min} - z2_{min} - dx_{min}$

Experiment design

Each test run is a mimicking usage of duodenoscope usage while systematically controlling the product and usage variables. In each test run, only one variable is tested by means of the groups (Table 6). In between each test run, all stainless steel construction parts are first cleaned, then disinfected (70% ethanol) and finally disassembled and sterilized (at 121±1°C for 50 minutes). After sterilization of the construction parts, the dirty side and the clean side basin are immediately covered by the cover to prevent cross-infection of the basins and then assembled. Also, the table was disinfected (70% ethanol), the construction set-up was assembled using disinfected gloves and the sterilized O-rings (at 121±1°C for 50 minutes) were installed in the axle grooves using sterile tweezers.

Prior to the test run, the seals' airtightness was tested by pressurizing the dirty side basin to 240 mbar with the leakage tester. Then, the test runs were started by inserting BHI 500µL BHI broth in the dirty basin. Each test run was 60±2.5 minutes: the maximum time of an ERCP procedure.[29] The test runs were performed up to a sample size of at least 9 seal tightness tests per group. To reduce the influence of construction deviations in each of the 3 mockups of the construction set-up, both the axle-in-block and blocks-in-framework were randomly assembled in a controlled manner (Table 5 and Figure 3). As negative controls, test runs were done while having one mockup immersed with fresh BHI broth in both the clean and dirty basin and by performing the experiments on a block without borehole while following the test run protocol. After a test run, the clean side of the seal was sampled using fresh BHI broth by indirect immersion of the clean basin. The clean basin cover was lifted and 600µL BHI broth was inserted in the basin. The broth is refluxed (i.e. pipetting fluid in the basin and pipetting it out of the basin) for 10 times. After 200µL BHI broth of the clean side, the contents of the basin are transferred with a pipette to separate TSA plates. Each basin was sampled with a new pipette tip to prevent cross-contamination.

Table 4. Stretch and effective compression measured in the Olympus TJF-Q180V and in the experiments' reproductions. (min=minimum dimension with correction of tolerances and measurement accuracies and max=maximum dimension with correction of tolerances and measurement accuracies) (*=directly measurable in the construction and **= calculated using formulae of *Table 3*)

Dimension	Symbol	Measured in Olympus TJF-Q180V	Design for reproductions	Actual reproductions with		
Inner diameter of O-ring* (mm)			$d1_{min}$	2,07	2,30	2,30
			$d2_{max}$	2,13	2,32	2,32
Cross section of O-ring* (mm)			$d1_{min}$	0,47	0,55	0,55
			$d2_{max}$	0,53	0,57	0,57
Piston diameter*(mm)			$d9_{min}$	3,02	3,04	2,96
			$d9_{max}$	3,08	3,06	2,99
Bore diameter* (mm)			$d4_{min}$	3,17	3,19	3,19
			$d4_{max}$	3,23	3,21	3,21
Housing inside diameter * (mm)			$d3_{min}$	2,37	2,39	2,28
			$d3_{max}$	2,43	2,41	2,37
Radial housing depth** (mm)			t_{min}	0,37	0,39	0,41
			t_{max}	0,43	0,41	0,47
Diametrical stretch inside diameter O-ring installed** (mm)			s_{min}	0,24	0,07	0,00
			s_{max}	0,36	0,11	0,07
Percentage of inside diameter stretch**			S_{min}	11	3	0
			S_{max}	17	5	3
Percentage of O-ring cross-sectional reduction resulting from diametric stretch** (%)			R_{min}	6,62	2,30	0,00
			R_{max}	9,22	2,79	2,30
Cross section installed O-ring** (%)			$d2_{min}$*	0,43	0,53	0,54
			$d2_{max}$	0,49	0,56	0,57
Percentage of effective O-ring cross-section compression** (%)			C_{min}	0,00	23	13
			C_{max}	25	30	28

Table 5. Controlled assembly-randomization of the construction parts. Top: axle-in-block. Bottom: blocks-in-framework

Block# Run	Block 1	Block 2	Block 3
1, 4, 7, 10	Axle 1	Axle 2	Axle 3
2, 5, 8, 11	Axle 2	Axle 3	Axle 1
3, 6, 9, 12	Axle 3	Axle 1	Axle 2
Location in framework Location	**Left**	**Middle**	**Right**
1, 4, 7, 10	Block 1	Block 2	Block 3
2, 5, 8, 11	Block 2	Block 3	Block 1
3, 6, 9, 12	Block 3	Block 1	Block 2

Table 6. Four groups of the systematic investigation of bacterial tightness of reproductions of the distal sealing mechanism of duododenoscopes

Group Independent variable	1. Inactive use	2. Pressurization of dirty side	3. Rotating axle	4. Rotating, translating axle
Axial displacement of axle (mm)	0	0	0	0.375
Pressure at dirty side (mbar)	0	16	0	0
Frequency of 90°-dynamic rotation (per test run)	0	0	30	30

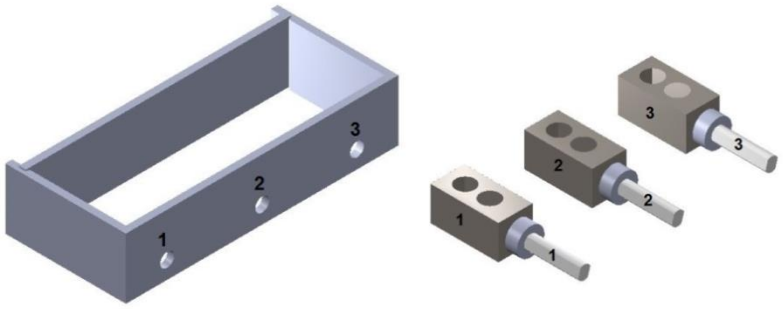

Figure 8. Controlled assembly-randomization of the construction parts. Left: axle-in-block. Right: blocks-in-framework

The plates are incubated for 25±0.25 hours at 36±1°C. The CFU on each plate were counted. *K. Pneumonia* on TSA is a flat CFU with a relatively large diameter, with a viscous/mucoid appearance and a yeasty odor. All colonies were checked on their appearances, and those being suspicious for not being *K. Pneumonia* were identified using MALDI-TOF MS (Bruker Nederland BV).

Data interpretation

All CFU of bacteria strains were identified using MALDITOF-MS, and stains other than K. Pneumonia were excluded from the results. These bacteria have a high likelihood to be cultured as a result of environmental contamination (environmental flora or dermal flora) during sampling, and therefore not counted as penetrated bacteria.

Statistics

The hypothesis was tested with a two-way Pearson Chi-Square test of independence while using 95% confidence interval (α-level= 0.05) assuming non-parametric matched data and sufficiently large sample size. The statistical tests were performed to analyze whether bacterial tightness ('bacterial penetration' = 1 and 'bacterial tight' = 2) and usage variables ('variables zero'= 1 and 'variable as in use' = 2) are independent of one another.

Disclosure statement

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Contents

Abstract

O-ring sealing constructions are widely used in medical instruments to prevent the loss of a fluid or gas in a construction by obstructing flow through the glands in the interface of two or more separate parts. Regarding O-ring used in medical devices, a classification may be made, namely O-ring seals (1) in direct contact with patient material and (2) in indirect contact with patient material. Especially for O-ring seals in direct contact with patient material, biocompatibility and microbiological safety are important to guarantee. To assure biocompatibility of a seal's material, the label USP class IV must be present. Regarding microbiological safety, exogenous cross-infections must be prevented; infections from microbes originating from other than the patients' own flora transmitted by another person or object.

Duodenoscopes are flexible endoscopes used to treat abnormalities in the biliary tree, a procedure also known as endoscopic retrograde cholangiopancreatographic procedures (ERCP). As a tool for physicians to guide instruments, such as biopsy needles, guide wires and stents, in the ducts, duodenoscopes are distally equipped with a forceps elevator steerable by an elevator wire. Unlike models of duodenoscopes sold before 2009, updated model duodenoscopes have an O-ring sealing construction in this distal elevator mechanism to prevent patient material from invading into the elevator wire channel. The air tightness of this distal O-ring seal is tested by several leakage tests during cleaning and disinfection of the duodenoscope.

The aim of this study was to evaluate bacterial leakage of airtight O-ring seals in direct contact with patient material while using as case study the distal seal in duodenoscopes. The increasing number of duodenoscope-related infections associated with multi-drug resistant bacteria emphasizes the relevance to determine whether an airtight O-ring seal also implies a microbial tight seal for this instrument. The study starts with an elaboration on O-ring seals in medical instruments including a systematic research on specific O-ring seals associated with adverse infection-safety events. Then, the study continues by focusing on duodenoscopes only. The air- and fluid tightness of the O-ring sealing construction was theoretically evaluated. Also, the bacterial tightness of the distal sealing construction was evaluated by a series of two individual studies: surveillance of bacterial leakage through the seal during repair and a systematic investigation studying bacterial tightness of airtight mockups of the distal sealing mechanism in duodenoscopes.

Two unique medical instruments have been associated with infection-safety events related to O-ring seals. Bacterial leakage has been found in static O-ring seals in dental implants in vitro, suggesting that bacteria can penetrate an O-ring seal. Static O-ring seals in dialyzers have been linked to infected patients, thereby concluding that O-rings might remain contaminated while disinfected. However, in all cases, the air- and fluid tightness of these apparent microbe-containing seals were not evaluated.

Based on the dimensions measured in one Olympus TJF-Q180V duodenoscope, it was theoretically evaluated that the diametrical stretch of the distal O-ring in these instruments is 11 to 17%; higher than the 1 to 4% recommended in literature. On the other hand, the effective compression seems to be in coherence with the recommended dimensions; the measured 0 to 25% approaches the recommended effective compression for static-dynamic seals of 15 to 20%. Aside, it was found that the leakage test, performed in between reuse of duodenoscopes, likely may not be pressurizing the space adjacent to the distal O-ring seal; a basic computational model indicated that no fluid flow is present after 51% of the elevator wire channel at the final stage of the test. Bacterial contamination at the clean side of the distal seal in used duodenoscope was not detected during microbiological screening. Only three distally-sealed duodenoscopes were sent for repair during the study period. No microbiological contamination was found in the samples.

In the systematic investigation mockups of the elevator sealing mechanism of Olympus TJF-Q180V duodenoscopes were subjected to leakage tests and test runs. During the test runs, product- and usage variables -expected to influence microbial leakage- were systematically controlled in experiment groups. The product variable controlled was the mockups' seals' effective compression: for the lowly-compressed seals 0-13% and for the highly-compressed seals 13-28%. The usage variables controlled in the test runs are gauge pressure on the seals' dirty side (set on the intra-abdominal pressure of 16 mbar gauge pressure), dynamic rotational loading of the axle (set on

maximum elevator usage of 30 times a 90°-rotation movements) and translational motion of the axle (set on the maximum axial displacement of 0.375mm). Other product or usage variables were kept constant at the level observed in the sealing mechanism or at the level recommended in literature as optimal regarding sealing efficacy. The constructions with highly-compressed seals (91%) were more likely to be airtight (at 240 mbar) than with lowly-compressed seals (31%) since a significant interaction was found between these variables ($\chi^2(1, N = 40) = 11.46$, $p <= .05$). The constructions with highly-compressed seals were equally likely to have microbial leakage (10%) than with lowly-compressed seals (0%) in passive conditions, since no significant interaction was found ($\chi^2(1, N = 40) = 11.46$, $p < .05$). Seals submitted to gauge pressure were equally likely to have microbial leakage (9%) than passive seals (10%), since no significant interaction was found ($\chi^2(1, N = 21) = 0.00502$, $p < .05$). Seals with a dynamic rotational loaded axle were more likely to have microbial leakage (60%) than passive seals (10%). A significant interaction was found ($\chi^2(1, N = 20) = 7{,}41$, $p < .05$). Seals with a dynamic rotational loaded translational axle were more likely to have microbial leakage (67 %) than passive seals (10 %), since a significant interaction was found ($\chi^2(1, N = 19) = 6.54$, $p < .05$). However, seals with a dynamic rotational loaded axle with axial displacement were equally likely to have microbial leakage (67%) as seals with a dynamic rotational loaded axle (60%), since significant interaction was found ($\chi^2(1, N = 19) = 0.0905$, $p < .05$). The results of the systematic investigation indicate that airtight O-ring seals can leak bacteria, even in static conditions. Moreover, rotation of the axle is a significant microbe-influencing variable.

Since the experiment set-up is a replication mimicking the distal sealing mechanism in duodenoscopes with high reliance, the results suggest that bacteria leak through the distal seal if the forceps elevator is extensively used. The best-case sealing conditions were used for the test runs, with the worst-case usage variables, therefore the result of this controlled investigation is an overestimation of the bacterial tightness during maximum use intensity. However, these conclusions need some reluctance, since the mockups' design and dimensions are not fully identical to that of the duodenoscopes' elevator mechanism.

It is important for medical designers, manufacturers and safety controllers to be aware that airtight O-ring seals in direct contact with patient material in reusable instruments may leak bacteria. Theoretically, exogenous cross-infections could appear, if microbes may leak through a seal, while killing of those microbes at the 'clean side' is not feasible and migration back to the patients' side is possible. Microbe-leaking O-rings may especially be a problematic design-related factor for medical instruments that are reprocessed with chemical disinfection. Follow-up studies are recommended for further investigation to the bacterial penetration of airtight O-ring seals with ISO 3601-2 complying sizes.

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Picture is made by Ilona Brouwer and Huub Scholing

Disclosure

This study was carried out under the supervision of the UMCG and the TU Delft. Required information was gathered from analyzing publications, instruments belonging to the UMCG and the TU Delft and contacting Rescope BV. No contribution was made by any manufacturer or other third party.

The results of this project could, but should not, be used to make conclusions about products of manufacturers, since no bacterial leakage was found in seal mechanisms with the exact design and dimensions of their products. If a reliable conclusion must be drawn about the bacterial tightness of O-ring seals in duodenoscopes, a study could be performed including actual duodenoscopes. The results of this study can be used as an initial awareness of possibly bacterial leakage in medical instruments in direct contact with patient material.

This report is intended for publication after an embargo valid for one and a half year after the publication date. Only the writer is responsible for the study methods, and generated results and conclusions.

Definitions

Bacterial leakage	Passage of bacteria through an obstruction that is intended to seal
Bacterial penetration	Migration of bacteria through one side of a barrier to the other side
Bacterial tightness	Degree of obstruction of the passage of bacteria between a clearance in a construction them
Product variable	Measurable variable that is defined by the design and manufacturing of a product
Cross-infection	An infection resulting from pathogenic microorganisms transferred from another person, object or location within the body
Endogenous infection	An infection resulting from pathogenic microorganisms from a patient's own microbial flora
Exogenous infection	An infection resulting from pathogenic microorganisms from environmental sources (i.g. healthcare workers, medical instruments)
Infection safety	Condition of being safe from becoming infected
Leak tight	Fully obstructing the passage of substances between a clearance in a construction
Leak tightness	Degree of obstructing the passage of substances between a clearance in a construction
Leakage test	Procedure intended to determine the leak tightness of a seal
Leakage	Passage of substances through an obstruction that is intended to seal
Microbial leakage	Microbial leakage may be defined as the passage of microorganisms (bacteria, mycobacteria, viruses, spores) through an obstruction that is intended to seal them
O-ring	A torus-shaped (e.g. ring with a circular cross-section) gasket or seal used compressed between separate parts prevent leakage in the construction
Patient material	Organic bio-material originating from patients, containing proteins and most likely micro-organisms, and therefore potentially infectious
Pseudo-infection	The presence of pathogenic microorganisms in absence of an actual infection
Seal	A part or substance that is used to join separate parts of a construction together in such a way to prevent them from coming apart or to prevent anything passing between them
Usage variable	Measurable variable that is induced by the usage of a product

Abbreviations

AER	automatic endoscope reprocessor
CDC	Center for Disease Control
CFE	colony-forming units
CRE	carbapenemase-resistant Enterobacteriaceae
CRKP	carbapenemase-producing *Klebsiella pneumoniae*
E. coli	*Escherichia coli*
ERCP	endoscopic retrograde cholangiopancreatography
EtO	ethylene oxide
EUS	endoscopic ultrasound
FDA	Food and Drug Administration
GI-endoscope	gastrointestinal endoscope
HLD	high-level disinfection
ISO	International Organization for Standards
K. pneumonia	*Klebsiella pneumonia*
LLD	low-level disinfection
MAUDE	Manufacturer and User Facility Device Experience
MDR	Medical Device Report
MDR bacteria	multi-drug resistant bacteria
MDRO	multi-drug resistant organism
P. aeruginosa	*Pseudonomas aeruginosa*
SAL	sterility assurance level

Symbols

In this report the definitions used for dimensions of O-ring constructions with matching symbols are directly copied from those in ISO 3601-2.[1]

bx	width of the O-ring housing
C	percentage of effective O-ring cross-section compression
*d*1	O-ring inside diameter
*d*2	O-ring cross-section diameter
*d*3	housing inside diameter for piston application
*d*4	bore diameter for piston application
*d*9	piston diameter
E	diametrical clearance
f	housing radius
g	extrusion gap / radial clearance
h	height of seal housing
M	bearing length
R	percentage of O-ring cross-sectional reduction resulting from diametrical stretch
S	percentage of inside diameter stretch
z	length of lead-in chamfer
M	bearing length from the bore opening to the
z1	angle of lead-in chamfer

Figure 1. O-rings are available in a wide range of sizes and material types

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Chapter 1. Introduction

1.1. Tightness of O-ring seals in contact with patient material

1.1.1. O-ring seals in medical instruments

O-rings are doughnut-shaped rings molded from elastomers or -less commonly- thermoplastic materials or metal, available in a wide range of sizes (*Figure 1*). O-rings are used for sealing to prevent the loss of a fluid or gas in a construction by obstructing the flow of fluids or gasses through the glands in the interface of two or more parts (*Figure 2 top*).

Different material types are available for O-rings; they can be selected based on material properties most suitable for its application. Only medical grade materials are allowed for use in medical instruments in contact with patients. This biocompatibility can be recognized by the label USP Class IV materials.

1.1.2. Infection prevention in reusable medical instruments in contact with patient material

During a procedure, medical instruments can become heavily contaminated with body fluids, such as blood, bile or intestinal fluid, also known as patient material. To prevent cross-infection between patients due to pathogenic micro-organisms in this patient material, the external surfaces of medical instruments accessible for patient material are cleaned and disinfected or sterilized (e.g. reprocessed) before reusing the device.[2]

Post-surgery-related infections can either be endogenous or exogenous. While endogenous infections are caused by patients' own microbial flora, exogenous infections are induced by micro-organisms from the external environment. Reprocessing of the medical instruments reduces the chance on exogenous cross-infections.

[3] In 2016 the ECRI's (Emergency Care Research Institute) Health Technology Hazard number one spot in the top 10 is improper cleaning and disinfection of flexible endoscopes. [4]

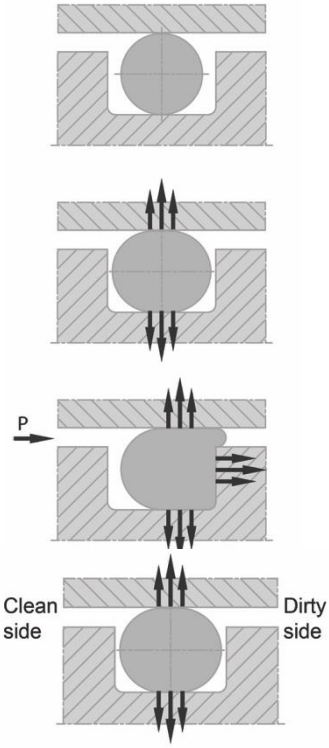

Figure 2. Cross-section of an O-ring construction.

Top: no compression.

Middle-top: primary sealing.

Middle-bottom: secondary sealing.

Bottom: sealing with a clean and dirty side.

1.1.3 Tightness of O-ring seals in contact with patient material

An O-ring seal construction consists of an O-ring in a gland in its interface: sealing is obtained by compression of the O-ring material in its housing. Primary sealing is provided by the material's elasticity (*Figure 2 middle-top*). Secondary sealing could be obtained if the O-ring construction is pressurized from one side (*Figure 2 middle-bottom*). The design of the O-ring housing defines the seal tightness, the performance of the seal to block fluids and gasses. Even seals for well-designed housings, leakage through O-rings is assumed to be present in small amounts; fluid- and gas leakage can be reduced to a level acceptable for its application.[5], [6]

Based on the interaction with the patient, O-ring seals may be divided into two groups: O-ring seals in direct contact with patient material and in indirect contact with patient material. O-ring seals in direct contact with patient material prevent the loss of fluids and gasses being patient material (e.g. blood, airway gasses, intestinal fluid and urine) in a construction. The dirty side of the O-ring encounters patient material and the other side of the O-ring stays clean because the flow of patient material is obstructed (*Figure 2 bottom*). O-ring seals in indirect contact with patient material prevent the loss of non-patient originating fluids or gasses (e.g. tap-water, tap-air) in a construction.

O-ring seals in reusable medical instruments in direct contact with patient material another kind of leakage -aside from fluid and gas tightness- is of importance; leakage of microorganisms. If the one side of an O-ring is freely accessible for patient material, leakage of micro-organisms to the other side of the O-ring must be prevented, especially when the housing is not reprocessed and when migration of microorganisms back to the patient side of the O-ring is possible. If not, micro-organisms can be transmitted between patients, and cross-infections safety cannot be guaranteed.

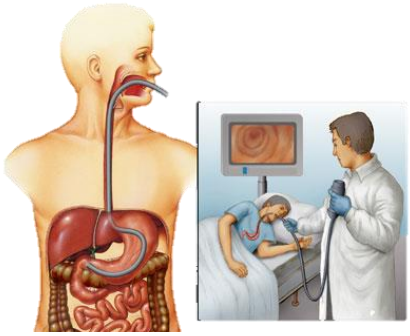

Figure 3. An endoscope is used to inspect the inside of the patient's body cavities

1.1.4. O-ring seal in duodenoscopes in direct contact with patient material

A duodenoscope is a specific type of flexible endoscope. Medical devices are designed to serve different clinical needs; diagnostic, surgical or therapeutic. In this context, endoscopes are special medical devices: they are designed to serve all three needs. The word '*endoscopy*' is a composition of two Greek words; '*Endon*' means '*inside*' and '*Skopeo'* means '*to look at'*, with a combined meaning '*looking inside*'. An endoscope is a medical device designed to enable the physician to look inside body cavities (*Figure 3*). [7]

Endoscope's shafts can be either rigid or flexible. A flexible endoscope has a long, small diameter shaft, and a control unit with knobs for bending of the distal tip with a camera and light source (*Figure 4left and right*). In the shaft different components are integrated; a fiber optic system and channels for irrigation, suction and influx of water and/or air and an instrument channel. [7] All these options of the flexible endoscope enable physicians to perform minimally invasive procedures; the physician has the possibility to perform diagnostic and therapeutic endoscopic procedures at the operation site without the need of invasive techniques. The operation site can be visualized by using the camera and light source on the distal tip combined with a computer. The operation site can be reached by steering the bending tip with manipulating the bending knobs and the operation site can be operated by inserting instruments, air and water at the control unit, leading through the channels to the opening in the distal tip.

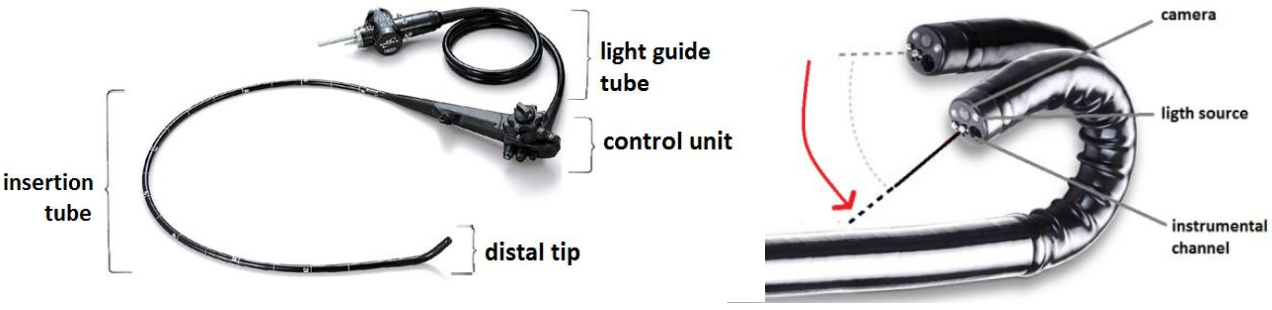

Figure 4. Left: A typical flexible endoscope: an instrument with a small diameter shaft with integrated fiber optic system and channels. Right: A typical endoscope has a bendable tip with camera, light source(s) and instrument channel outlet. Adapted from [65], [66], [67]

A duodenoscope is the type of flexible endoscopes especially adapted to perform endoscopic cholangiopancreatography (ERCP) procedures in the biliary tree. The biliary tree refers to the system of the pancreas, gallbladder and bile ducts. The pancreas and gallbladder produce bile, used for the digestion of food, what is collected and transported by the biliary three ducts. These ducts secrete its content in the upper part of the intestines directly situated after the stomach, also known as the duodenum. The biliary tree is accessible with from the duodenum. A duodenoscope is inserted in the throat and reaches, through the esophagus and the stomach, the distal part of the duodenum where the major duodenal papilla, a conjunction of the common bile duct and the main pancreatic duct, is situated (*Figure 6*). From this position, the physician is able to perform an ERCP procedure. The intra-abdominal pressure is raised to inflate the duodenum for optimizing the sight. Since the duodenoscope is a sideways viewing endoscope; with the camera and light source at the side of the tip, the major duodenal papilla can be visualized from the duodenum.

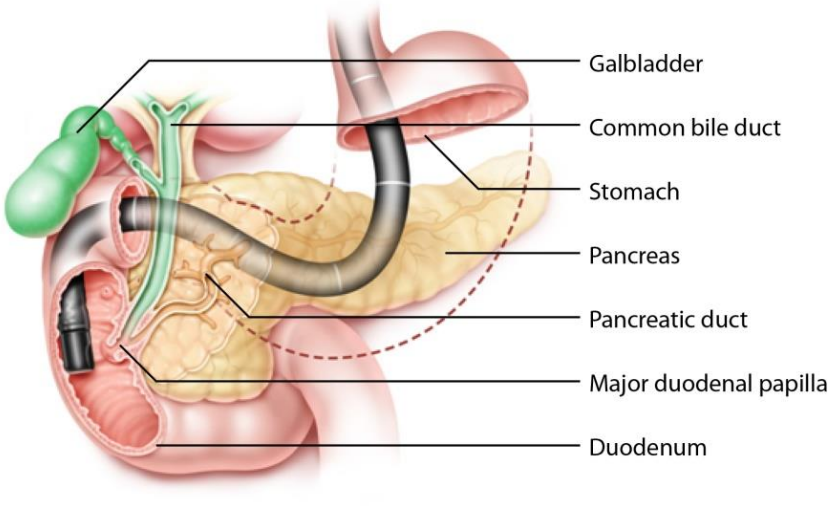

Figure 6. A side-ways viewing duodenoscope is used to perform an ERCP procedure from the duodenum in the pancreatic and bile ducts. The forceps elevator at the tip is used to bend endoscopic accessories, for example biopsy forceps, into the major duodenal papilla. [68]

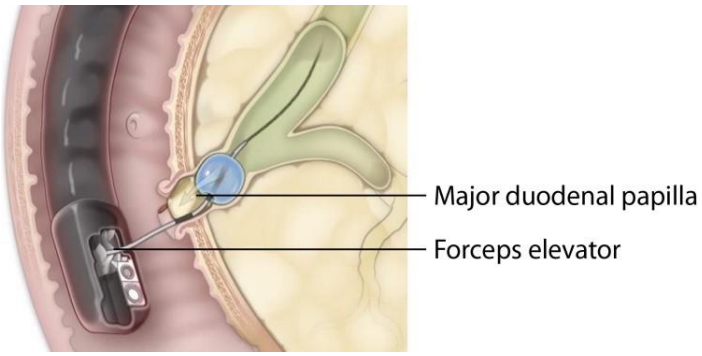

Figure 5. The forceps elevator at tip of a duodenoscope can bend instruments into the major duodenal papilla during ERCP procedures [99]

To be able to guide surgical instruments in the major duodenal papilla, duodenoscopes have a special feature at the tip: a forceps elevator, which is controlled by a wire adjustable with a knob on the control unit (*Figure 5*). Instruments can be inserted through the instrument channel and the forceps elevator provides the ability to bend these instruments sideways into the major duodenal papilla.

Duodenoscopes are sold by three different manufacturers; Olympus with a market share of 85%, Pentax with a market share of 12% and Fujinon with a market share of 3% (*Table 1*). All the three manufacturers have duodenoscopes in two models: an older model, and a new model –first introduced in 2009- with some design updated adaptions. From the design point of view, the updated model differs from the previous on two aspects; the channel which guides the elevator wire is now sealed instead of freely accessible, and the distal cap is fixated instead of detachable. In the before-2009 sold models the elevator wire channel needs to be reprocessed since patient debris can enter the elevator wire channel during ERCP procedures. Instead, the updated model duodenoscopes have a shielded elevator wire channel by means of an O-ring seal at the distal tip.

1.1.5. Infection prevention in reuse of duodenoscopes

Reprocessing guidelines are optimized to achieve a minimum risk of exogenous infections by microbial contamination on flexible endoscopes; this risk was in 1993 associated with one in 1.8 million endoscopes. [8] However, in 2014 the claim has been made that this figure is only a tip of the iceberg: exogenous infections could easily remain unrecognized or they could be underreported.

A senate member of the United States analyzed the number of patients infected by MDRO associated with contaminated duodenoscopes. In 2012, the first patients with updated-model-duodenoscope-related MDRO infections were reported, with 81 cases, and the years after patients were reported too. The infections were coagulated in outbreaks and the outbreaks were each in a different hospital spread over Europe and the United States (*Figure 7*).

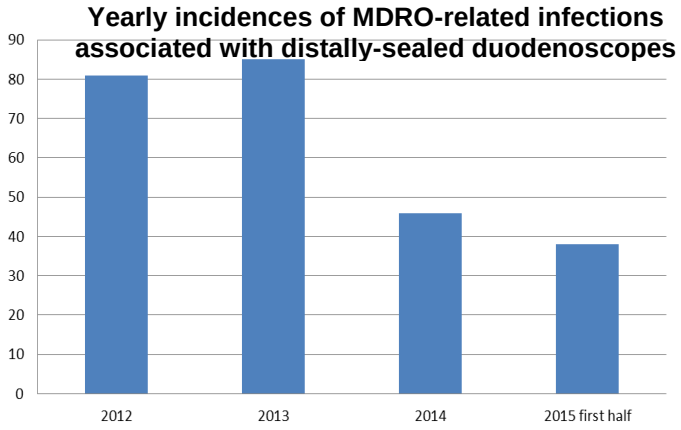

Figure 7. The incidence of duodenoscope-related MDRO infections by closed model duodenoscopes in the period of 2012 till June 2015 per year. Data used from [5]

In the literature review performed prior to this, the risk of patient infection by microbial contamination on duodenoscopes has been calculated to be one in 7.555 to 14.539 patients undergoing ERCP procedures. This recalculated risk is between 124 to 234 times higher than the 'golden standard' infection risk of 1 in1.8 million that was associated with gastrointestinal endoscopes in 1993.[9]

The incidence of MDRO duodenoscope-related infections per manufacturer, with for Olympus 197 (79%), for Pentax 47 (19%) and for Fujinon 6 (2%), can be directly related to their market share with for Olympus 85%, Pentax 12%, Fujinon 3% (*Table 1*). [10], [9]

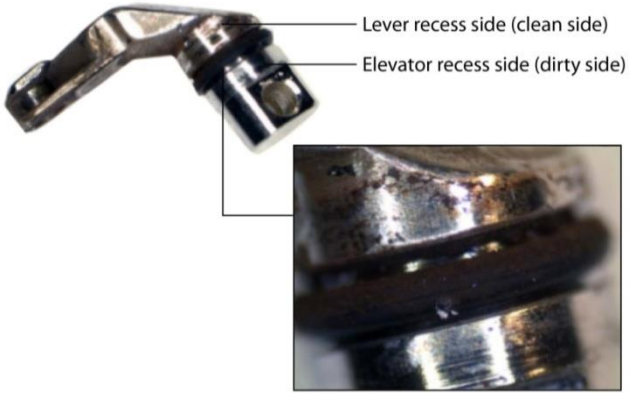

Figure 8.Microscopical images of the elevator mechanism and the O-ring seal. Brownish debris is clearly visible at the non-patient side of the seal, at the clean non-patient side of the housing and is slightly visible at the dirty patient side of the O-ring. This debris could be a result of leakage of the O-ring. Adapted from [11]

The complexity of the design of the updated models of duodenoscopes has often been suggested as the cause of the apparent increase of duodenoscope-related infections. The duodenoscopes and endoscopic ultrasound endoscopes (EUS-endoscopes) have a more complex tip design comparing to other flexible endoscopes due to its forceps elevator mechanism, resulting in more reprocessing steps to comply with. The updated model duodenoscopes of the manufacturers Olympus, Pentax and Fujinon have less reprocessing steps, however, the residual steps seem more prone for the persistence of patient contamination comparing to the open model duodenoscopes and EUS-scopes, due to the fixed distal cap that restricts accessibility for brushes and fluids during reprocessing.[9] Moreover, it has been suggested that the O-ring sealing mechanism in the distal tip of updated 'closed' model duodenoscopes might be leaking or trapping microorganisms during use, and thereby acting as a reservoir impossible to reach during the preprocessing process.[11], [12]

In 2012 an investigation of an Olympus TJF-Q180V, the updated model duodenoscopes of the manufacturer Olympus, was published; a persistently contaminated duodenoscope was systematically disassembled and microbiologically evaluated.[11] The O-ring seal housing in the elevator construction located in the distal tip the O-ring had, aside from clearly visible wear, brownish debris on its surface. The O-ring and the non-patient side (i.e. clean or side) of the housing was highly covered

Table 1. The relative reported MDRO-infection per market share for each manufacturer

Manufacturer	Market share	Reported incidence MDRO-infections (total incidence of 250 patients)	Relative reported incidence of MDRO-infection per market share [9]
Olympus	85%	197 (79%)	0.93
Pentax	12%	47 (19%)	1.58
Fujinon	3%	6 (2%)	0.66

with the debris, also some debris was visible at the patient-side of the O-ring. The brownish debris can most likely be explained by leakage of fluids from the dirty patient side to the clean shielded side of the O-ring seal (*Figure 8*).

In January 2016, manufacturer Olympus had to recall their closed model duodenoscopes. Healthcare facilities owning Olympus TJF-Q180V were asked to return it to their local service point; with the goal to replace the seal in the tip for a tighter one. The FDA clears that the design modifications are 'intended to reduce infection risk', because by modifying 'its design of the elevator channel sealing mechanism to create a tighter seal' will lead to 'reduce the potential for leakage of patient fluids and tissue into the closed elevator channel'. [13] According to Olympus the replacement is an improvement by 'controlling the tolerances more closely'.[14]

1.2. Problem definition and aim

Even though O-rings are considered as an effective seal for fluids and gasses, this does not necessarily have to imply that the construction seals for micro-organisms too. No indication has been found in during the studies in this thesis that the Medical Device Certification (CE and FDA) have included the requirement for manufacturers to evaluate O-ring seals on microbial sealing efficiency; the infection safety of O-ring construction in direct contact with patient material seems like a 'grey area' and therefore its contribution to duodenoscope-related infections cannot be determined. Designers, manufacturers, and auditors should have knowledge about safe reuse of O-rings in dynamic construction in direct contact with patient material, especially with the rise of MDRO's.

Duodenoscopes are widely sold medical instruments with as dominating manufacturer Olympus. The distal O-ring construction in the distal tip of duodenoscopes is used as an obstruction to prevent flow of patient material and reprocessing fluids into the elevator wire channel. This way, the dirty side of the O-ring construction - the elevator recess – is freely accessible for patient material and the other side of the construction – the elevator lever recess can be considered as the clean side. As quality control measure, the performance of the distal seal is tested by air leakage testing. But aside from being air- and fluid-tight, the construction also needs to be bacterial tight to ensure infection safety; once microorganisms originating from patient material are transferred to the clean side, they are entrapped during cleaning and disinfection, meaning that they will not be removed. In that case, safe reuse cannot conclusively be assured, since the entrapped microorganisms might be transferred back to the patient side again.

The aims of this thesis were to (1) determine the frequency of infection safety adverse events related to O-ring seals in medical instruments, (2) to indicate if the O-ring seal in the elevator mechanism of duodenoscopes is indeed tight for fluids and gasses, and (3) to indicate if airtight O-ring seals are tight for bacteria when they are in direct contact with a bacteria-containing fluid.

1.3. Study approach

Since O-ring sealing constructions are made in a wide variety of sizes and materials, first the studies in this thesis should focus on relevant cases for the evaluation of microbial tightness of seals. Therefor the O-ring sealing construction in the elevator construction located in the distal tip of duodenoscopes is used as the study case. Olympus has gained 85% of the total market share of duodenoscopes, therefore the Olympus TJF-Q180V is used for the evaluation for the O-ring seal in the elevator mechanism of duodenoscopes. Olympus' recall for the replacement of the distal O-ring

seal was announced after finishing the methodology of this thesis, the studies focus on pre-recall Olympus TJF-Q180V duodenoscopes.

All the following chapters in this thesis have the aim to partially answer the research question *'What is the bacterial tightness of the O-ring seal in the distal tip of pre-recall Olympus TJF-Q180V duodenoscopes?*'. An overview of the chapters and their corresponding secondary research questions is given (*Table 2*). Each chapter presents a separate conducted study, therefore first the secondary research question and, if applicable tertiary research questions are presented, then the methods and results are discussed, concluding with a discussion of how the results (partially) answer the research question. In the final chapters, the sub-answers are combined to answer the main research question.

In 'Part A' infection safety adverse events related to O-ring seals in medical instruments are mapped. In Chapter 2 a systematic search is presented indicating the reported- infection safety adverse events related to O-ring seals in medical instruments. In Chapter 3 it is elaborated how the O-ring seal in the elevator construction located in the distal tip of duodenoscopes could contribute to endangering infection safety. In 'Part B' the air and fluid tightness of the O-ring seal in duodenoscopes is theoretically analyzed; in Chapter 4 a theoretical analysis will indicate whether the O-ring seal in the elevator construction located in the distal tip is designed according to the standards of O-ring seal dimensioning, and in Chapter 5 the effectiveness of the leakage test during manual cleaning on the O-ring seal is analysed with a simple computational model. In 'Part C' the bacterial tightness of the O-ring seal in duodenoscopes is evaluated by two separate studies: Chapter 6 describes surveillance study sampling the dirty and clean side of the distal tip of duodenoscopes after disassembling during maintenance and Chapter 7 describes a systematic investigation of bacterial tightness through airtight O-ring seals of mockups of the elevator construction of Olympus TJF-Q180V duodenoscopes in test runs mimicking usage.

Table 2. Overview of the secondary research questions

Part	Chapter	Secondary research question
Part A. Infection safety adverse events related to O-ring seals in medical instruments	Chapter 2. Reported-infection safety adverse events related to O-ring seals in medical instruments	What infection safety adverse events have been reported related to O-ring seals in medical instruments?
	Chapter 3. Elaboration on the distal O-ring seal in duodenoscopes	How can the O-ring seal in the elevator construction located in the distal tip of duodenoscopes contribute to endangering infection safety?
Part B. Air and fluid tightness of the distal O-ring seal in duodenoscopes	Chapter 4. Theoretical analysis of the air and fluid tightness of the distal seal	Is the O-ring seal construction in the distal tip of a pre-recall Olympus TJF-Q180V designed according to the standards of O-ring seal dimensioning?
	Chapter 5. Computational analysis of the (air) leakage test during manual cleaning	What is the pressure on the O-ring seal in the elevator construction in duodenoscopes during the leakage test in the manual cleaning phase?
Part C. Bacterial tightness of the distal O-ring seal in used duodenoscopes	Chapter 6. Screening of microbial contamination leaked through the seal during maintenance	Is the clean side of the O-ring seals in duodenoscopes contaminated with microbes?
	Chapter 7. Systematic investigation of the bacterial tightness of airtight seals	Are airtight distal O-rings of Olympus TJF-Q180V duodenoscopes also tight for bacteria?

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Part A. Infection safety adverse events related to O-ring seals in medical instruments

Chapter 2. Reported-infection safety adverse events related to O-ring seals in medical instruments

2.1. Introduction

2.1.1. Relevance of systematic search for reported adverse events

O-rings are frequently used in mechanical constructions as sealing solution since they are low in costs and efficient in sealing. Medical grade O-rings are widely sold by several manufacturers for many types of medical instruments. According to the O-ring manufacturer *Apple Rubber Product*, their medical grade O-rings are sold for medical appliances, dialyzers, medical pumps, intravenous components, feeding devices, implant materials and seals for non-implantable instruments.[15] The manufacturer *Eriks* states that their medical grade O-rings are used for medical applications in autoclaves, medical- and diagnostics instruments and pumps. [16] Even though O-ring manufacturers claim to sell their medical grade O-rings worldwide for a wide range of applications, an overview of their application in medical products is hard to make: O-rings are installed inside a housing and are thereby, most often, 'hidden' in construction.

2.1.2. Aim

The aim of this chapter is to make an overview of the reported infection safety AEs related to O-ring seals. This will reflect the current state of knowledge of infection safety of O-rings in medical instruments.

The secondary research question will be answered '*What infection safety adverse events have been reported related to O-ring seals in medical instruments?'.*

2.2. Methods of the systematic search

Databases examined are *Scopus* and *PubMed*. The search line used is a combination of the keywords '*(o-ring OR gasket) and (infect* OR outbreak OR contaminat*) and (bacter* OR micro*)'.* Articles are included with the following criteria;

- The reported problem with the described O-ring must be associated with a microbiological safety issue;
- The described O-ring seal must have been used in a reusable medical instrument.

The results are reported in an overview of these characteristics: reusable medical instrument type, sealed fluid, type of contact with patient material, description of the microbiological contamination, and mechanical properties of the construction.

2.3. Search results

The search resulted in 103 results via Scopus and 27 results via PubMed, of which five passed the selection criteria. These five relevant results described two unique reusable medical instruments with AEs of O-rings infection safety. These reusable medical instruments are dialyzers and dental implants. An overview of the reported infection safety adverse events related to O-ring seals in medical instruments is given (*Table 3*).

Dialyzers are medical instruments intended to function as an artificial kidney by removing detrimental elements from the blood, also known as hemodialysis. In 2005, 40% of all centers used multi-use dialyzers that require cleaning and disinfection prior to reuse. The dialyzers consist out of different blood compartments and to prevent leakage between these compartments and its header an O-ring is

used functioning as a gasket (*Figure 9.left*). *Jones et al. (1970)* have reported that bacteria have been found on the O-ring and its groove after reprocessing. [17] In order to facilitate cleaning and disinfection, some types of dialyzers have screw band headers for disassembly. *Bland et al. (1989)* have reported a model type on which bacteria have been found on the O-ring and its groove, even though the dialyzer compartments could be disassembled prior cleaning and disinfection,. [18] *Flaherty et al. (1993)* have reported that patients after a dialyze treatment were infected by a bacteria strain also cultured from the O-ring in these dialyzers. [19] *OYong et al. (2013)* describe an outbreak among three patients, all three underwent hemodialysis with the same dialyzer, and had the same genotypic analysis of a bacteria strain (e.g. with corresponding genes) in their blood cultures. This indicates a common source of transmission and the O-ring was hypothesized as a source of the infections. [20] All the studies, except *OYong et al.,* have reported that the problem with bacteria in the blood compartment was solved with disinfection of the O-ring or replacing it for a new O-ring. [17]–[19]

Most dental implants have two pieces: the abutment, functioning as the dental root, and the tooth implant functioning as the new teeth (*Figure 9.right*). The most undesired complication after dental implantology is peri-implantitis: inflammation of the hard or soft tissue surrounding the dental implant. [21] The void of the implant-abutment-interface can be a reservoir for bacteria which may induce the development of peri-implantitis. The influence on bacterial leakage by applying seals between the implant and abutment has been studied for over more than twenty years. *Nayak et al. (2014)* have analyzed the efficacy of an antibacterial sealing gel in comparison with an O-ring by analyzing bacterial leakage in vitro. Sterilized, assembled dental implants were immersed in a bacterial suspension for 120 hours. After thoroughly washing and disinfecting the outer surfaces, the implants were disassembled and bacterial leakage through the O-ring was evaluated by culturing the inner surfaces of the implant. While microbial growth was seen in both groups, the O-ring group had 20 times more leakage bacterial leakage; the O-ring-sealed group had with 72,55±63,63 colony forming units (CFU) per milliliter significantly more leakage than the gel-sealed group with 3,18±3,46 CFU per milliliter. [21]

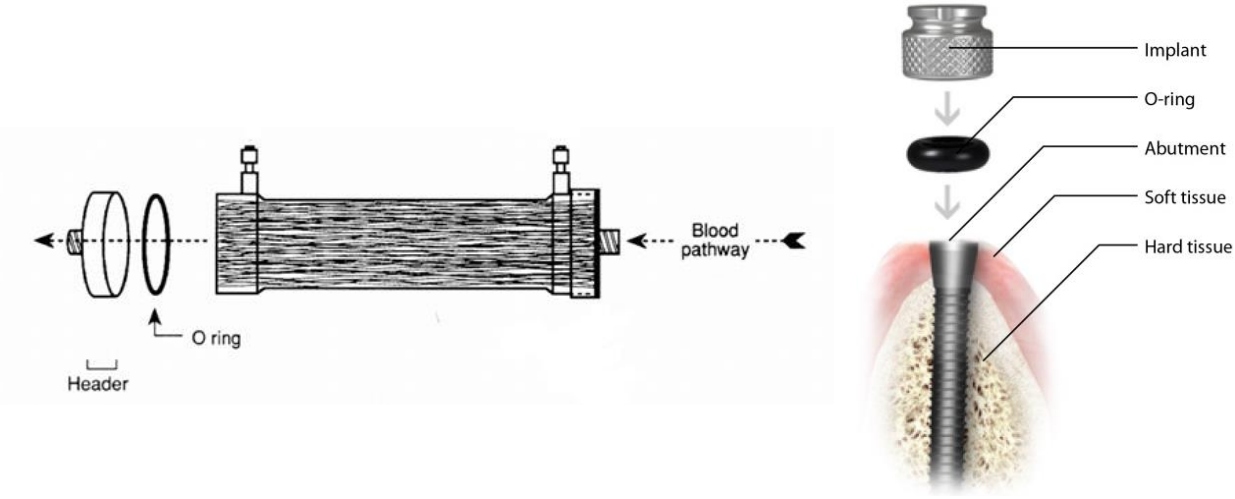

Figure 9. Abstract drawings of medical instruments reported with infection AEs related to O-ring. Left: dialyzers adapted from [19]. Right: dental implant, adapted from [69].

2.4. Discussion and conclusions

'What infection safety adverse events have been reported related to O-ring seals in medical instruments?'

The results of the systematic search indicate that two medical instruments have been associated with infection safety-related adverse events. Bacterial leakage through an O-ring seal has been reported in dental implants after 120 hours, which may indicate that O-rings can leak bacteria in static conditions. However, the gas or fluid tightness is not measured for these seals, and therefore a reference for the sealing efficacy is not present (i.e. the seals might as be improperly designed or manufactured). Next to dental implants, in dialyzers O-rings and its housing have been reported to be contaminated even after the standard reprocessing processes. Despite fact that in these studies it is not verified if the reprocessing is conducted as intended, this AEs may indicate that O-ring construction can be problematic to effectively reprocess.

The results could be considered as a reflection of the current state of knowledge of infection safety of O-rings in medical instruments. However, most likely these reported adverse events are not a reflection of the actual safety of O-rings in medical instruments; most likely it shows only 'a tip of the iceberg' [22]–[24] since exogenous cross-infection infections can be unrecognizable or remain underreported or unnoticed. [22]

Table 3. Overview of reusable medical instruments with O-ring seals associated with an AE

Medical instrument	Sealing fluid	Patient material contact	Sealing construction	Year	Total articles
Dental implant	Saliva, dental pulp	Direct	Static	2014	1 [21]
Dialyzer	Blood	Direct	Static	1970, 1989, 1993 and 2014	4 [17]–[20]

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Chapter 3. Elaboration on the distal O-ring seal in duodenoscopes

3.1. Introduction

3.1.1 Duodenoscopes in clinical use

Flexible endoscopes are available in a range of sizes; the length of the insertion tube varies from 700 to 2200 mm, the diameter of the insertion tube varies from 3.8 to 15 mm and the diameter of the instrument channel varies from 0.6 to 4.8 mm.[9] The dimensions of the shaft of a type of flexible endoscope are optimized for the performance of a procedure in a specific body part. Duodenoscopes with an insertion tube with a length of 1250 mm and a diameter 7.5 to 12.1 mm, and an instrument channel diameter of 2.0 to 4.8 mm.

Abnormalities treated during ERCP are: biliary tract diseases such as strictures, bile stones, malignant and benign biliary strictures, and sphincter dysfunction by placing a stent or performing a small surgery, and pancreatic duct leaks.[25] ERCP procedures are often accompanied with fluorescence to create an image. The contrast agent is inserted into the ducts, proving radiological visualization of the pancreatic and biliary three ducts. Using the fluoroscopic images any present abnormalities can be detected and is in most cases also threatened during the procedure. In 2009, the number of performed ERCP procedures was estimated at 14 in 10.000 patients.[26] From 1984 to 2009, the number of diagnostic ERCP's declined from 7.28 to 1.11 procedures in 10.000 humans and therapeutic ERCP's rose from 0.42 to 12.75 procedures in 10.000 humans. [26]

Post-ERCP-related AEs occur with an average rate of 5.3%. [27] The most common post-ERCP AE is pancreatitis: infection of the pancreas. In a large population-based cohort study, post-ERCP-related pancreatitis has been reported at 2.4%, still, the reported prevalence of this AE is a wide range; a systematic literature review has reported a range from 1.60%-15.7%. [28] Other AE are cholangitis (infection of the bile ducts) and hemorrhage, with a complication rate of respectively 1.5% and 1.4%. The most lethal post-ERCP AE is blood stream infection, also known as sepsis. The patient mortality rate is 25% for sepsis with antibiotics-sensitive bacteria and 50% for sepsis with multi-drug resistant micro-organisms (MDRO).[29] Post-ERCP-related sepsis adverse event rate varies among different studies between 0.25% and 5.4% (*Table 4*). [30], [28], [31] Endoscopy-related infections are most often associated with endogenous infections. [30] Endogenous infections cannot be controlled by the reprocessing procedures. On the contrary, exogenous infections can be prevented by effective reprocessing.[30]

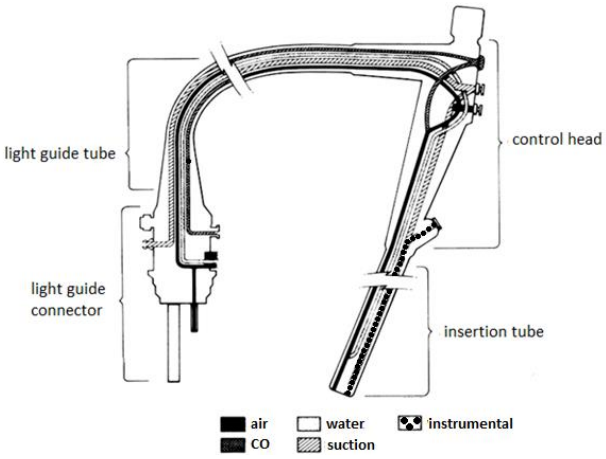

Figure 10. The different parts of a flexible endoscope and the channels. Adapted from [70]

Table 4. Most common post-ERCP-related AE's and the adverse event rate. Population based-cohort study (16.855 patients) [28]

Adverse event	AE-rate
Pancreatis (i.g. infection of pancreas)	2.4%
Cholangitis (i.g. infection of bile ducts)	1.5%
Hemorrhage (i.g. bleeding due to ruptured blood vessel)	1.4%

3.1.2. Reuse of duodenoscopes

Reprocessing of the flexible endoscope reduces the chance of post-endoscopy related exogenous infections. To prevent endogenous cross-infection between patients due to pathogenic micro-organisms in this patient material, all external surfaces of reusable medical instruments need to be reprocessed before reusing the device. During reprocessing, the number of micro-organisms on a flexible endoscope is reduced to a safe level by reprocessing; a procedure of cleaning followed by high-level disinfection (HLD). [32]

All the channels accessible for patient material in flexible endoscopes need to be reprocessed to remove and kill the microorganisms and other waste elements from the channels, before reuse (*Figure 10*). In the updated model duodenoscopes, this channel is closed by a seal in the tip. This reduces the reprocessing workload; reprocessing of the elevator wire channels is not needed.

3.1.3. Aim

The increase in reported duodenoscope-related infections since the introduction of the distally-sealed model duodenoscopes makes critics doubt about the infection safety of the O-ring seals in the elevator mechanism of duodenoscopes.[12] In this Chapter in-depth insight is created of the design reprocessing method of this feature. The aim of this Chapter is to state a clear understanding about how O-ring seals in the elevator mechanism of duodenoscopes could pose a risk of infection safety.

In this Chapter the secondary research question will be answered *'How can the O-ring seal in the elevator construction located in the distal tip of duodenoscopes contribute to endangering infection safety?*'. This is done using the following tertiary research questions:

- *'What is the design of the O-ring seal in the elevator mechanism of duodenoscopes?'*
- *'How is the O-ring seal in the elevator mechanism of duodenoscopes reprocessed?'*

3.2. Methods of the semi-systematic search

The design of the distal seal in duodenoscopes is explained using renders of a self-constructed model of the distal tip of duodenoscopes in SolidWorks Education Edition 2014-2015. The input for the geometry of the model are references resulting elaborating on duodenoscope's design [11], [33] a visit to flexible endoscope repair center Rescope BV at Beuningen (the Netherlands) and disassembly of an Olympus TJF-Q180V (Chapter 4.2.1. Dimensions of the O-ring sealing construction in the distal tip of duodenoscopes). The reprocessing of the distal tip of duodenoscopes is elaborated by detecting deviating steps for duodenoscopes from the standard reprocessing procedure for flexible endoscopes. In order to do this, reprocessing manuals of the duodenoscope manufacturers.[34]–[37], [38]

3.3. Design of the distal seal

The forceps elevator in the distal tip can be controlled by the control knob on the control unit, interconnected by a long wire: the elevator wire (*Figure 12-top*). The knob is connected to an elevator rod, which is soldered to the proximal ending of the elevator wire while the distal ending is connected to the forceps elevator. The elevator wire in distally-sealed models is indirectly connected to the forceps elevator; it is interconnected by separate part: the elevator lever (*Figure 120bottom, Figure 13*). The forceps elevator and the elevator lever are together functioning as one part; the axle of the elevator lever is fitted in a bore of the elevator while a tight connection is created by fastening the forceps elevator on the axle of the elevator lever by means of a glued screw fixation. The axle of the elevator lever is supported in a borehole in the partition between the elevator recess and the elevator lever recess. Therefore, manipulation of the forceps elevator functions as a lever construction; pressure exerted on the elevator control knob manipulates the forceps elevator; by controlling the elevator control knob, the elevator wire moves in proximal or distal direction creating a (resp.) pulling or pushing force on the cantilevered forceps elevator, creating a moment which rotates the axle of the forceps elevator, (reps.) opening or closing the forceps elevator (*Figure 12-bottom*). [39]

The forceps elevator and the elevator lever rotate both in a separate recess (*Figure 13*). The elevator recess is openly accessible for patient material and reprocessing fluids. The elevator lever recess is separately covered with a lid underneath the distal cover. In the lever axle, at the location of the partition, an O-ring in installed in a groove (*Figure 13-bottom*). The radial O-ring is compressed between a borehole in the partition and the lever's axle. This mechanism seals the elevator wire channel while it still allows rotational movement of the axle of the elevator lever.

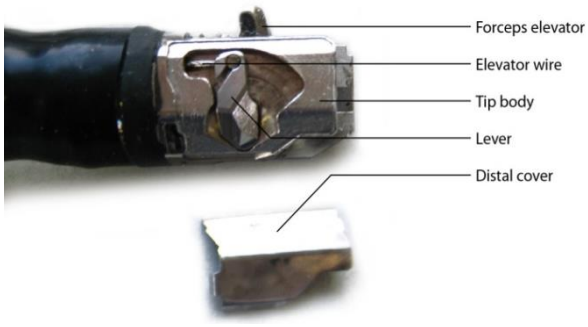

Figure 11. A side view of the distal tip at the side of the elevator recess without distal cover and with a disassembled lever recess cover.

An overview is listed of the models of therapeutic duodenoscopes used in clinics (*Table 5*). According to the FDA, the sealing in the distal tip of duodenoscopes is either with a single or a double (two in parallel) O-ring seals at the lever axle. For Olympus and Fujinon it has been reported that the seal consists of a single O-ring. For Pentax could not be confirmed whether a single or double seal is applied.

Table 5. The properties of different models of therapeutic duodenoscopes used in hospitals

Manufacturer	Accessibility of the elevator wire channel	Model name	Number of O-rings in sealing mechanism in distal tip
Olympus	Freely accessible	TJF-145, TJF-160(V)	0
	Distally-sealed	TJF-Q180V	1[11]
Pentax	Freely accessible	ED34-i10T	Information has not been found
	Distally-sealed	ED-3490TK or ED-3670TK	1 or 2 [33]
Fujinon	Freely accessible	ED-200XE, ED-420XL and ED-450XT [40]	0
	Distally-sealed	ED-250XT5, ED-250XL5, ED-450XT5, ED-530XT[40]	1 [10], [40]

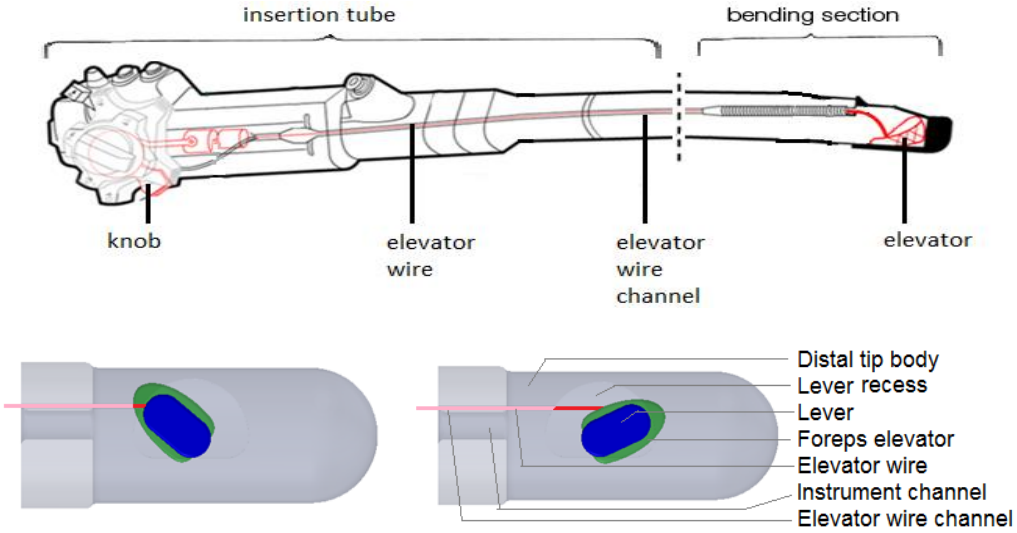

Figure 12. The elevator wire channel conducts the elevator wire (in red) to lift the forceps elevator. Top: the elevator can be manipulated by the control knob. Bottom: the elevator wire channel has an elevator cleaning channel in distally-sealed model duodenoscopes, and therefore is indirectly connected to the forceps elevator by a lever. Adapted from [52]

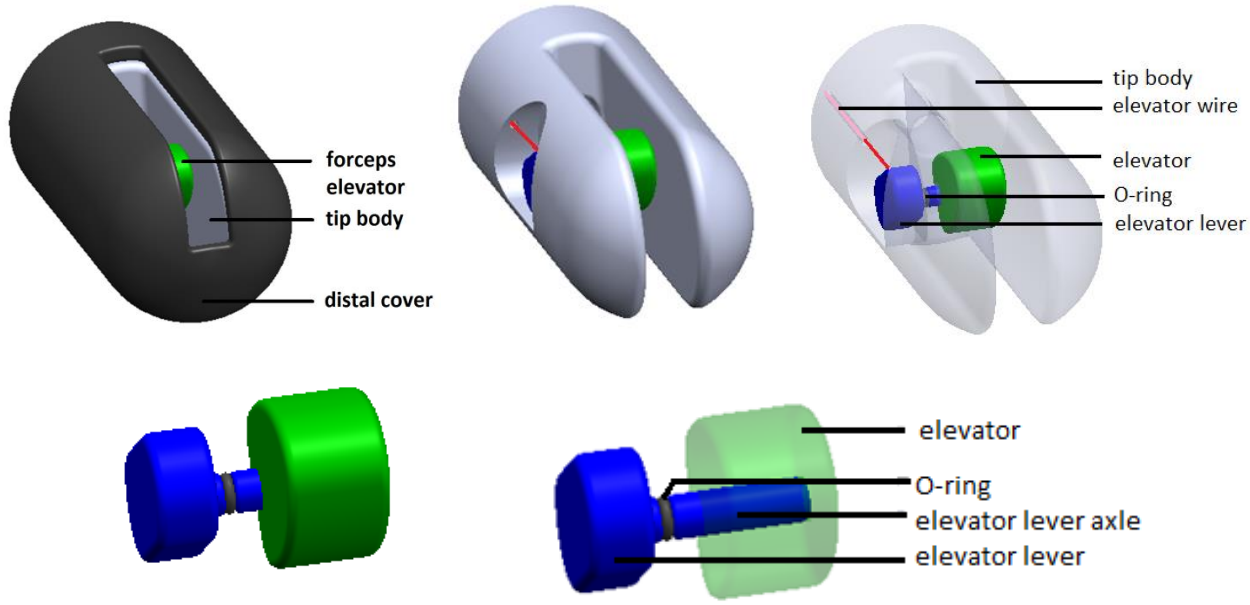

Figure 13.Perspective drawing (isometric projection) of the distal tip of duodenoscopes. Top-left: distal tip with distal cover. Top-middle: complete distal tip without lever recess cover and without distal cover. Top-right: transparent tip body. Left-bottom: without distal cover. Right-bottom: without distal cover and with transparent forceps elevator. Three plane projections of the distal tip and of each part separately are given in attachment 1.

3.4. Cleaning and disinfection of the distal tip

3.4.1. Cleaning and disinfection applicable for all flexible endoscopes

The reprocessing process of flexible endoscopes consists of multiple steps (*Figure 14*). Effective reprocessing has on average a reduction of 10 logs. [41],[42],[43] Effective reprocessing is in The Netherlands evaluated by rinsing sterile water through the channels after a full reprocessing cycle; if it contains less than 20 colony forming units per 20 milliliters bacteria the flexible endoscope is effectively reprocessed.[37]

Before reprocessing, gastrointestinal endoscopes may be contaminated with 1 million bacteria.[41], [44] In order to yield effective reprocessing results, cleaning of flexible endoscopes must be thorough, since HLD is a relative reduction (6 logs) of microorganisms (*Table 6*). Therefore, flexible endoscopes reprocessing starts in the operation room with pre-cleaning: internal surfaces are rinsed. Next, the flexible endoscope undergoes manual cleaning. Cleaning is defined as the removal of visible soil [32]; all the loose parts of the device (i.e. valves, distal covers) are disassembled and brushed, the outside is wiped and rinsed and lumina are cleaned with disposable brushes for at least 3 times with an enzymatic detergent.

The next step is high-level disinfection (HLD). The high-level disinfectant used for HDL varies among countries; most commonly used are peracetic acid or glutaraldehyde. The disinfectants must come in contact with instrument surfaces for specified contact times and temperatures. The HLD phase is most frequently facilitated by an automatic endoscope reprocessor (AER), which often also repeats the cleaning phase with rinsing the lumen and outer surface to improve cleaning results. [45], [46] Endoscopes are dried after reprocessing in a drying cabinet to create detrimental circumstances for waterborne bacteria. [45],[47]

Gasses and fluids are shielded from invading into the endoscope's; all channels, covers, valves and connections on the exterior need to be leak tight for patient material. Permanent seals are made of kit or glue, for example, the distal cover is glued on the distal tip in closed model duodenoscopes, and the connection of the distal tip to the bending section to the connection of the instrument insertion opening to the shaft is made by kit. To ensure the functioning of the channels and seals, leakage tests are performed during reprocessing; the interior space of the endoscope is pressurized with an air

compressor connected to the leak tester valve.[37], [48] If the flexible endoscope is fully immersed in water, a leak can be detected by detecting visible air bubbles escaping from the endoscope surface (through the channels or seals). Also, a pressure drop could be observed at the pressure indicator. Generally, a flexible endoscope with a leak is sent to a repair center.

Table 6 The reported level of micro-organisms on GI endoscopes after reprocessing [41], [42], [43] . *SAL=sterility assurance level*

Average number of bacteria HLD	Reduction cleaning	SAL after cleaning	Reduction of HLD	SAL (average number bacteria) after HLD
10^5 to 10^9	4 logs	10^1 to 10^5	6 logs	10^{-3} to 10^0

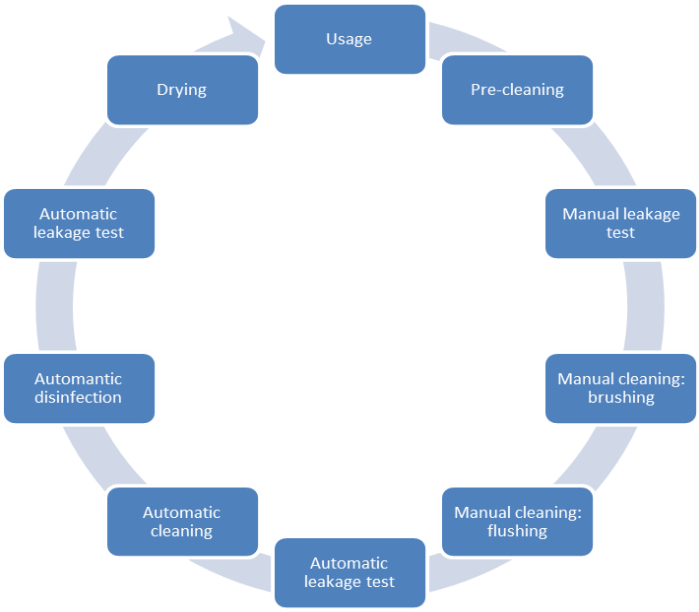

Figure 14. The steps of reprocessing of flexible endoscopes

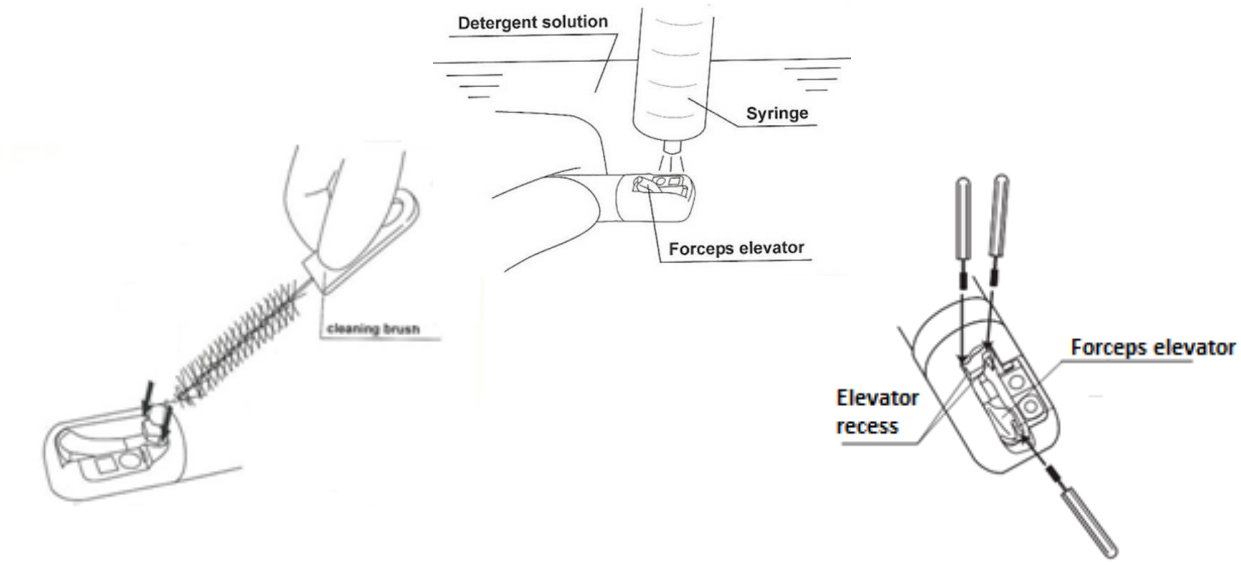

Figure 15. Additional reprocessing steps for duodenoscopes. Bottom-left: the forceps elevator and elevator recess need to be brushed. Bottom-right: the forceps elevator and elevator recess additionally need to be brushed with a small brush. Top: forceps elevator and elevator recess need to be flushed with a syringe. Adapted from [34], [38].

3.4.2. Cleaning and disinfection applicable for duodenoscopes only

Duodenoscopes' procedure for automatic cleaning and disinfection is almost identical as for other types of flexible endoscope. For manual cleaning of duodenoscopes additional need to be performed, due to the forceps elevator in the distal tip. The surfaces of the elevator and of the elevator recess need to be brushed and flushed with a cleaning detergent solution during manual cleaning (*Figure 15-bottom-left*). First, those surfaces need to be cleaning by brushing the elevator and elevator recess with a large cleaning brush. The elevator must be put in several positions to be able to brush all areas. After, the surfaces of the forceps elevator and the elevator recess needs to be brushed with an additional small brush (*Figure 15-bottom-right*). Finally, all surfaces need to be forcefully flushed with a detergent solution using a 30-mL syringe while immersing the distal tip in the solution (*Figure 15-top*).

During the leakage test, also the leak tightness of the distal O-ring seal is tested; the interior of the duodenoscope is pressurized through the leakage test connection with an inflow pressure of 240 mbar. [37], [48] The closed elevator lever recess, adjacent to the O-ring seal, is openly accessible for airflow from the pressurized main chamber; an airflow pathway exists from the main chamber through the elevator channel -through a small opening at the proximal end of the elevator wire channel of about 1 to 2 mm- mounting into to elevator lever recess (*Figure 16*).

Microbiological monitoring of the reprocessing effectiveness of duodenoscopes is advised to do every week. The cleanliness of the distal tip is not separately evaluated; the final rinsing water of the instrument channel is collected at the instrument channel opening in the distal tip also running past the elevator and its recess. [37]

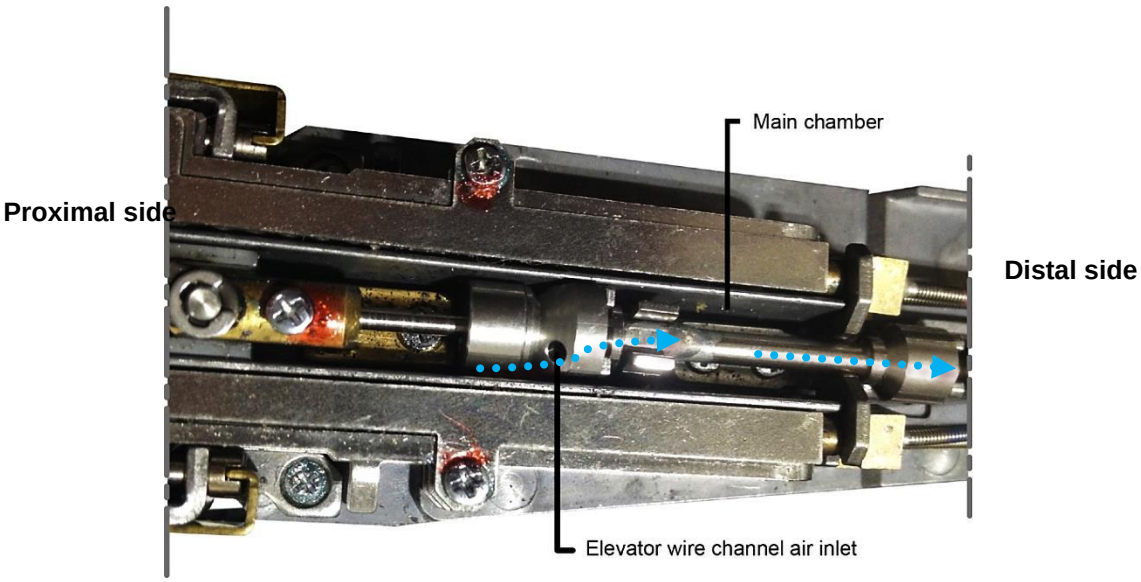

Figure 16.Picture of the interior of an Olympus TJF Q180V duodenoscope: the about 1-to-2 mm opening in the elevator wire channel is visible. Air flows through the inlet into the elevator wire channel (dotted blue arrow). Picture was taken at Rescope BV in Beuningen in The Netherlands.

3.5. Discussion and conclusions

'How can the O-ring seal in the elevator construction located in the distal tip of duodenoscopes contribute to endangering infection safety?'

The O-ring seal in the elevator mechanism of duodenoscopes has the advantage that it cancels out the requirement of reprocessing of the elevator wire channel. Olympus' and Fujinon's distally-sealed models duodenoscopes have a single O-ring seal and this information has not been found whether Pentax has a designed their distally-sealed model with a single or double O-ring. In the distally-sealed duodenoscopes, patient material can only access surfaces up to the radial O-ring seal; this splits the elevator mechanisms into two sides: the dirty side, freely accessible for patient material, and the clean side, sealed with patient material.

Patient material is free to access the elevator recess up to the O-ring (*Figure 17*). Theoretically, patient material can be stuck in the clearance gap, on the O-ring surface and in O-ring housing and might leak through the O-ring to the elevator recess (*Figure 17*). All these surfaces may then act as a micro-reservoir trapping bacteria, other micro-organisms and for example proteins. The presence of microorganisms in this micro-reservoir is not likely to be detected during microbiological surveillance since only the instrument channel is sampled by a collection of the final rinsing water. The contamination at the 'clean' side may leak back during ERCP procedures and cause exogenous cross-infections. Since the 'clean' side is unlikely to be cleaned and disinfected, contamination may remain unaffected over use cycles.

Based on estimation, the surfaces of the seal and the clearance at the patient's side seem unreachable for the small brush, therefor cleaning and disinfection need to result from fluid flow during reprocessing. The resulting flow velocity at the surface at these surfaces will be negligible, due to the small clearance between the borehole and the lever axle. In order to prove this analysis, a detailed flow analysis in a computational model could be made, to evaluate the exact fluid velocity at the surfaces of the dirty side of the O-ring during manual cleaning, automatic cleaning and automatic disinfection. However, the calculated fluid velocities cannot be benchmarked to a required standard, since the minimum level of fluid velocity for sufficient reprocessing results is undetermined. [49]

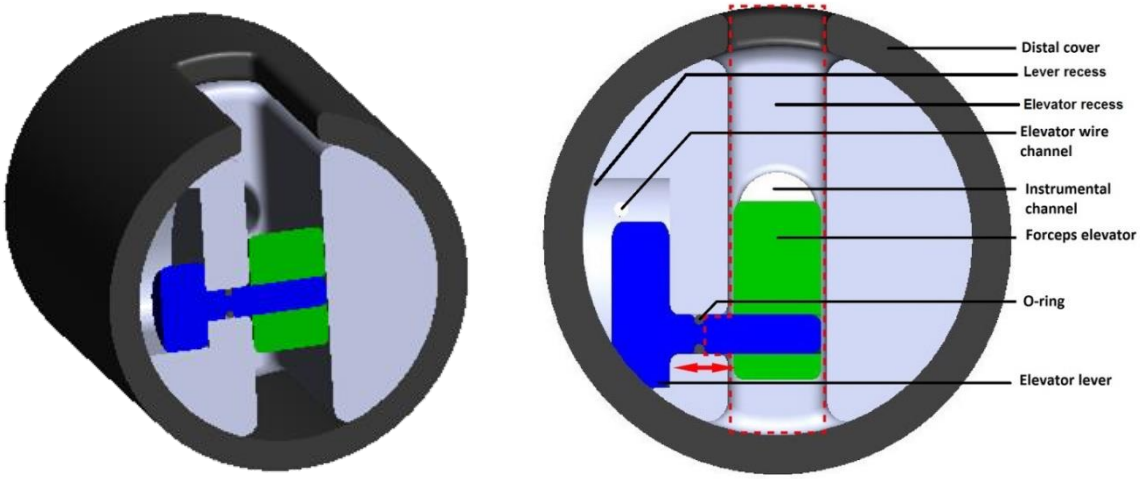

Figure 17. An abstract 3D-representation of the cross-section of the distal tip of distally-sealed duodenoscopes. The area's inside the red dashed line can be covered with patient material. If the O-ring may leak microorganisms, they may move in the direction of the arrows (back-and-forth from the elevator recess and the lever recess).

Part B. Air and fluid tightness of the distal O-ring seal in duodenoscopes

Chapter 4. Theoretical analysis of the air and fluid tightness of the distal seal

4.1. Introduction

4.1.1. O-ring application classification

O-rings can either be applied in static and dynamic constructions. Dynamic constructions are shafts in a supporting construction with a rotary, reciprocating or oscillating (e.g. both rotary and reciprocating) movement pattern. For static constructions both axial and radial installation can be used, for dynamic constructions, only radial installation should be used. Depending on the type of movement pattern, the O-ring groove must be located in the piston (i.g. inner sealing) or its housing (i.g. outer sealing). An overview is given of type of O-ring sealing type and their advised application (*Figure 18*). [50], [51] Depending on the seal application (*Figure 18*) and the dimensions of the to-be-sealed construction, the most suitable size can be chosen, either the cross-section as the inner diameter. Since the axle rotates during an ERCP procedure, if the forceps elevator is used, the O-ring seal in the new model duodenoscopes can be considered as a quasi-dynamic O-ring seal.

4.1.2. O-rings' sealing performance

Sealing performance is defined by the sealing interface: the contact of the O-ring material and its housing. The amount of contact between the O-ring material and its housing defines the sealing performance directly. On the contrary, material failure is crucial for sealing performance over time.

The geometry of the housing combined with the O-ring size determines the sealing surface because it defines stretch and effective compression of the seal. The stretch and effective compression must be optimized to have the desired sealing performance. The stretch must be minimized, since material under high stretch is known to have increased material failure. The effective compression needs to be balanced; seals with a high effective compression have a more stable sealing surface, whereas low effective compression results in less material failure as a result of lower friction. Increasing O-ring cross section can both have a beneficial or inconvenient effect on the sealing performance; a larger cross section gives more stability to the sealing surface meanwhile leading to more friction (i.g. material failure).

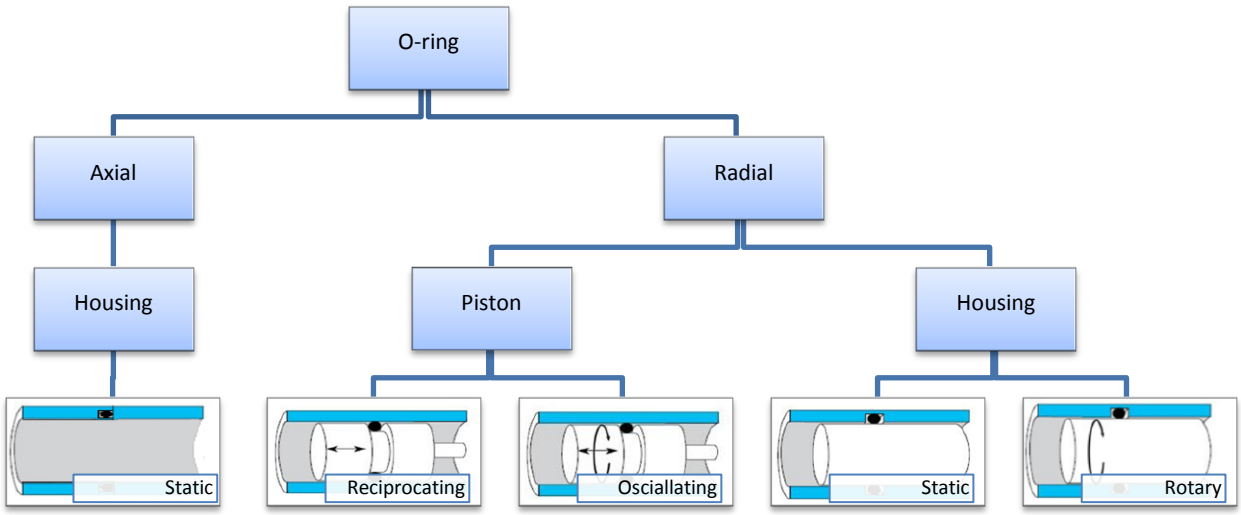

Figure 18. Overview of O-ring sealing types and their advised application

Additionally, other factors can influence the sealing interface. The installation method is important for material performance; therefore the construction must have lead-in chamfers and low surface roughness of the surfaces in contact with the O-ring is recommended. Finally, radial clearance must be in an acceptable low range, because it can cause seal extrusion and thereby material failure.

If the O-ring sealing construction is well designed and manufactured, it is considered as an efficient seal for fluids. However, handbooks warn for low amounts of gas leakage due to leakage of the O-ring material and the construction material interface and through the permeable O-ring material. To enhance the design and manufacturing of well-constructed O-ring seals, empirical-based recommendations are available. Normative recommendations are provided by the International Organization of Standards. Additionally, literature is available written by the dominating O-ring manufacturers Trellenborg, Parker, Apple Rubber Products and Eriks.

4.1.3. Standardized dimensions

ISO 3601 Part 1 compiles a list of metric sizes of O-rings including tolerances, in the inner dimension and the cross section. These metric sized O-rings have a wide range of varieties of the inner dimension of 0.74 to 658.88 mm and a cross section of 1.02 to 6.99 mm. [52] ISO 3601 Part 2 compiles a list of normative definitions of O-ring housings, and normative housing dimensions and design coupled with the normative O-ring sizes. However, these housing dimensions are only stated for fluid power systems, either hydraulic or pneumatic applications. The list of housing dimensions and design starts at the size of inner dimension 2.90 mm and the cross section of 1.78 mm. [1] For housing dimensions and design for O-rings smaller than 2.90x1.78 mm or for non-normative O-rings sizes, only the literature published by manufacturers can be consulted. [50], [51], [53], [54]

In the following chapters, the definitions and matching symbols used for dimensions of O-ring constructions are directly copied from those in ISO 3601-2.[1] The symbols are marked in the abstract representation of an O-ring, a piston and its housing (*Figure 19*). Below the copied list from ISO 3601-2 with the construction dimensions used in this report.

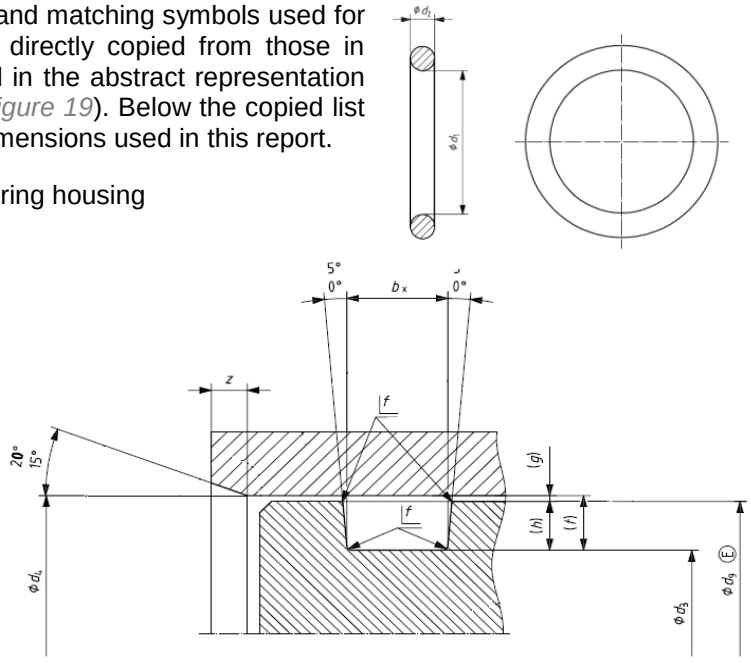

Figure 19 Dimensions with symbols of O-ring constructions with piston (i.e. inner) O-ring construction. Top: O-ring. Bottom: piston and housing. Adapted from [1]

a roughness of the side surface of the O-ring housing

*b*x width of the O-ring housing

C percentage of effective O-ring cross-section compression

d1 O-ring inside diameter

d2 O-ring cross-section diameter

*d*3 housing inside diameter for piston application

*d*4 bore diameter for piston application

*d*9 piston diameter

f housing radius

g radial clearance / extrusion gap

h height of seal housing

R percentage of O-ring cross-sectional reduction resulting from diametric stretch

S percentage of inside diameter stretch

z length of lead-in chamfer

Additionally, in this report definitions are used for dimensions of O-ring constructions with matching symbols which are not copied from ISO 3601-2.

M bearing length and *z1* angle of lead-in chamfer

4.1.4. Aim

The dimensions of this the distal O-ring sealing construction can be compared with the standardized dimensions recommended in the literature. Since those standardized dimensions are optimized to prevent air and fluid leakage, they will be used to theoretically evaluate O-ring seal performance in the distal tip of duodenoscopes.

In this Chapter, the secondary research question will be answered *'Is the O-ring seal construction in the distal tip of a pre-recall Olympus TJF-Q180V designed according to the standards of O-ring seal dimensioning?*'. This is done using the following tertiary research questions:

- *'What are the dimensions of the O-ring seal construction in the distal tip of a pre-recall Olympus TJF-Q180V duodenoscope?'*
- *'What should be the dimensions of the pre-recall Olympus TJF-Q180V duodenoscope according to the dimensions recommended in literature?'*

4.2. Methods

Only the factors were evaluated influencing the sealing surface evaluated (4.1.2. O-rings' sealing performance);

- Radial clearance (g);
- O-ring positioning (*Figure 18*);
- O-ring cross section (d2);
- Percentage of inside diameter stretch (S);
- Percentage of effective compression (C);
- Width of the O-ring housing (bx).

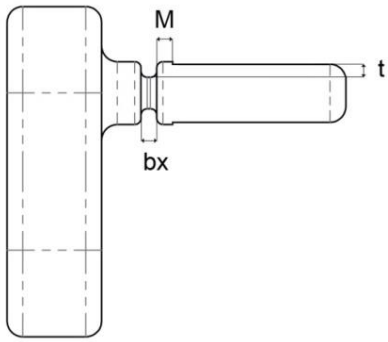

Figure 20. The elevator lever including the O-ring groove of the Olympus TJF-Q180V

The dimensions required to calculate these factors were measured in one Olympus TJF-Q180V. The values found in the elevator construction of the distal tip of duodenoscopes were compared with the dimensions recommended in the literature.

4.2.1. Dimensions of the O-ring sealing construction in the distal tip of duodenoscopes

For this study, only one disassembled Olympus TJF-Q180V was available. The dimensions of the O-ring and its housing were measured with a caliper with 0.03 mm accuracy (Skandia digital 150 mm). Dimensions measured were the piston diameter (d9), the bore diameter (d4), housing inside diameter (d3), width of the housing (bx) and the bearing length (M) of the O-ring housing, and the inside diameter (d1) and cross section (d2) of the O-ring (*Figure 19 and Figure 20*). With the values of these dimensions, the O-ring's inside diameter stretch (S) was calculated. Using this value, the percentage of effective O-ring cross-section compression (C) was calculated using the following formulae for the percentage of cross-sectional reduction of the O-ring (R).

Percentage of cross-sectional reduction O-ring.[1], [50] for a stretch range of 0-3%:

$$R_{0-3\%} = 0{,}01 + 1{,}06\,S - 0{,}1(S)^2$$

Percentage of cross-sectional reduction O-ring.[1], [50] for a stretch range of 3-25%:

$$R_{3-25\%} = 0{,}56 + 0{,}59\,S - 0{,}0046(S)^2$$

4.2.2. Search for standardized dimensions recommended in literature

It would be most ambiguous to compare the design of the distally-sealed O-rings construction in duodenoscopes with normative data of the ISO standards. Since the O-ring seal in closed model duodenoscopes is smaller than the smallest standardized O-ring size in ISO 3601-2, recommended dimensions were collected from literature provided by manufacturers Trellenborg, Parker, Apple Rubber Products and Eriks. The sealing surface influencing factors were evaluated for all these manufacturers.

4.3. Results

4.3.1. Dimensions of the distal O-ring sealing construction in duodenoscopes

The dimensions of the O-ring and its housing measured in the Olympus TJF-Q180V are listed in (*Table 7*). With the values of these dimensions, the stretch, effective compression and radial clearance were calculated (*Table 8*). A full movement of the elevator is estimated at maximally 90° (*Figure 21*).

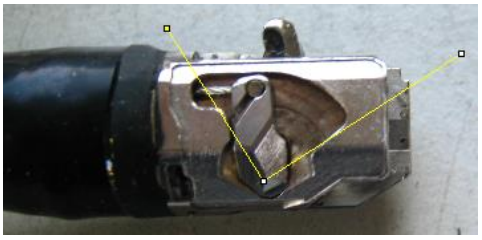

Figure 21. The elevator lever can freely rotate in its recess for max. 90°

Table 7. Dimensions including measurement accuracy of the O-ring construction in the Olympus TJF Q-180V

Items	Symbol	Dimension including measurement accuracy (mm)
Piston diameter	d9	3,05±0,03
Bore diameter	d4	3,20±0,03
Housing inside diameter	d3	2,40±0,03
Width of the O-ring housing	bx	0,80±0,03
Bearing length	M	0,65±0,03
O-ring inside diameter	d1	2,10±0,03
O-ring cross-section diameter	d2	0,50±0,03

Table 8. Stretch and effective compression of the O-ring construction in the Olympus TJF Q-180V. Minimum and maximum values calculated with the measured dimensions of Table 7 by considering the measurement accuracy.

Item	Symbol	Formula	Value
Radial clearance / Extrusion gap	g_{min}		0,075±0,06
	g_{max}		
Diametrical stretch inside diameter O-ring installed (mm)	s_{min}	$=d3_{min}-d1_{max}$	0,24
	s_{max}	$=d3_{max}-d1_{min}$	0,36
Percentage of inside diameter stretch	S_{min}	$=(s_{min}/d1_{max})*100[\%]$	11
	S_{max}	$=(s_{max}/d1_{min})*100[\%]$	17
Percentage of O-ring cross-sectional reduction resulting from diametrical stretch	R_{min}	$= 0,01 + 1,06(S_{min}) - 0,1 (S_{min})^2$	6,62
	R_{max}	$= 0,01 + 1,06(S_{max}) - 0,1 (S_{max})^2$	9,22
Cross-section installed O-ring (mm)	$d2^*_{min}$	$=d2_{min} - d2_{min}(R_{max}/100)$	0,43
	$d2^*_{max}$	$=d2_{max} - d2_{max}(R_{min}/100)$	0,49
Percentage of effective O-ring cross-section compression	C_{min}	$=(d2^*_{min}- t_{max})/d2^*_{min}*100[\%]$	0
	C_{max}	$=(d2^*_{max}- t_{min})/d2^*_{max}*100[\%]$	25

4.3.2. Standardized dimensions recommended in literature

The recommended ranges for radial clearance, stretch and effective cross-section according to literature are listed (*Table 9*).

For rotary seals, the smallest O-ring cross-section feasible is advised, in order to reduce friction of the O-ring material on its mating housing surfaces. However, for rotary shafts with a speed below 60 meters per minute the size of the cross-section is not critical. On the contrary, a smaller cross section is detrimental for sealing surface stability resulting in a less stable seal; it is more sensitive for tolerance stack-up, has a poorer compression set, is more sensitive for dirt and scratches and is more sensitive for displacements (*Table 10*). [50]

Even for rotary seal applications, a rod seal (i.e. housing sealing or outer sealing) is advised. Rotary O-ring seals have resulting centrifugal forces on an O-ring installed in a piston groove, resulting in pre-material failure due to rubbing surfaces of the O-ring in its housing.[50], [51]

4.4. Discussion and conclusions

'Is the O-ring seal construction in the distal tip of a non-recalled Olympus TJF-Q180V designed in accordance with to the standards of O-ring seal dimensioning?'

Using the standardized dimensions recommended in the literature, it can be determined if the dimensions in the Olympus TJF Q-180V are compliant with those recommended, and thereby if the construction is well-designed to prevent leakage of gasses and fluids (*Table 11*). The percentage of inside diameter stretch of the installed O-ring is between 11 and 17%, which is outside of the advised

Table 9. Radial clearance, stretch and effective compression according to literature

Dimension	Symbol	Value	Comment	Risk sealing contact loss	Risk material failure
Radial clearance (mm)	g	0,05-0,1	Valid for O-rings with 1,0-2,0mm cross section. For silicone, the values must be reduced by 50%[54]	None	If the clearance is large relative to the cross section, there is a risk of seal extrusion
Percentage of inside diameter stretch	S	1-4% [51], [54]	None	If the O-ring is stretched, the seal reduces in effective compression	High stretches can lead to premature failure
Percentage of effective O-ring cross-section compression	$C_{, dynamic}$	6-20% [50], [54]	Valid for cross sections above 1.8 mm. Must be higher for smaller dimensions (to compensate for tolerance stack up)	To low compression leads to leakage	None
	$C_{, static}$	15-30% [50], [54]		None	Too high compression leads to material destruction

Table 10. Effect of the cross section on O-ring sealing performance [50]

Sealing performance	Low O-ring cross section	High O-ring cross section
Increasing	Less sensitive for tolerance stack-up Better compression set Less sensitive to dirt and scratches More stable for displacements	Less friction
Decreasing	More friction	Sensitive for tolerance stack-up Poorer compression set Sensitive to dirt and scratches Less stable for displacements

range of 1-4%.A highly stretched O-ring can show a premature failure by material damage. The –in literature- advised range for dynamic seals of 6-20% and for static seals of 15-30% for seals with a cross section above 1.8mm. Since the seal in the Olympus TJF-Q180V is a quasi-dynamic seal, it would be convenient to have this value is inside both ranges, meaning that the allowable range would be between 15-20%. The percentage of effective compression measured in the duodenoscopes is between 0 and 25%. This range is too wide to be able to access the sealing performance based on the effective compression. Measuring the dimensions with a higher accuracy than the used ±0.03mm would yield a smaller range. The radial clearance is, with 0.075±0.03mm, within the advised range of 0.05 to 0.1mm. This advised range for radial clearance is valid for seals with a 1.0 to 2.0mm cross section; advice for seals with a lower cross section was not found. Also, according to *Eriks* the extrusion gap values for silicone O-rings need to be reduced by 50%, leading to a reduced range of 0.025-0.05. [54] Therefore, it is not possible to make a solid statement about the effects of the radial clearance on the sealing surface in distal O-ring seals in duodenoscopes. However, assuming the advised range mentioned, the radial clearance is expected to be acceptable, and thereby lowering the chance of premature material failure due to extrusion of the O-ring.

The O-ring position in the elevator mechanism is in the distal tip of duodenoscopes in the shaft. The estimated time of the shaft is minimally 1 second per full movement of the elevator. The rotary speeds of the shaft can be calculated using the piston diameter. The maximum speed of the rotary shaft of the elevator lever is 2.4 mm/sec (=144mm/min=0.144m/min). For rotary seals, it is advised to install the O-ring in the housing instead, to avoid rubbing of the O-ring on the surfaces due to centrifugal force due to the rotation, increasing the risk of leakage and material deconstruction. However, since the O-ring seal in the elevator mechanisms in the distal tip of duodenoscopes is quasi-dynamic with a low-speed rotary shaft, the 'mispositioning' of the O-ring is expected to have a very low impact or none. The used O-ring has with a cross-section of 0.5mm a lower size than the smallest standard size -of 1.78 mm- complying with ISO 3601. A low cross section has low friction during rotation of the construction. On the contrary, a higher cross section is more sensitive for tolerance stack-up, has a poorer compression set, is sensitive to dirt and scratches and is less stable for displacements comparing to higher cross sections. Thereby, the thin duodenoscope's O-ring probably must endure hardly any material failure due to low amounts of friction, however, is sensitive for leakage.

For future studies, more product variables could be measured to evaluate their possible contribution to leakage. For example, surface roughness has an influence on the level of sealing contact at the sealing interface and can thereby may influence leakage tightness [5]. A full list of factors that may influence microbial leakage related to the product is given below.

- Bearing length of the O-ring sealing construction [50]
- Radial clearance of the O-ring sealing construction [50], [55]
- Effective compression of the installed O-ring [50], [51], [54], [55]
- Hardness of the O-ring [50]
- Material of the housing [50], [51], [54], [55]
- Material of the O-ring [50], [51], [54], [55]
- Cross-section of the O-ring [5], [50]
- Stretch of the installed O-ring [50], [51], [54], [55]
- Surface finish the seal-contact-surface of the housing [50], [55], [5]
- Surface finish of the O-ring [50], [55], [5]

Table 11. Dimensions measured in an Olympus TJF-Q180V compared by the reference values according to literature

Item	Symbol	Measured	Advised
Radial clearance	g	0.069-0.081mm	0.05-0.1
Percentage of inside diameter stretch	S	11-17%	1-4%
Percentage of effective O-ring cross-section compression	C	0-25%	15-20%
O-ring positioning		Piston sealing	Not clear
O-ring cross-section diameter	d2	0,47-0.53mm	Large
Width of the O-ring housing	bx	0,77-0.83mm	0.80mm

Chapter 5. Computational analysis of the (air) leakage test during manual cleaning

5.1. Introduction

5.1.1. Leakage test of elevator wire channel seal

The tightness of flexible endoscopes is tested during the leakage tests at every reprocessing cycle, such that malfunction of the seals over the lifetime of the flexible endoscope should be detected. The first leakage test is done prior to cleaning, the second before automatic cleaning is started and the third after automatic disinfection. The leakage test is performed by compressing air in the interior of the flexible endoscope with an air compressor compatible with the endoscope, according to Olympus for two minutes.[34] For example, the leakage tester used in the UMCG is the Olympus Mu-1 air compressor configured at 240 mbar.

As explained in 'Chapter 3.4. Cleaning and disinfection of the distal tip ', the space before the O-ring seal, the elevator lever recess, should be pressurized by the influx of air through the elevator wire channel. The elevator wire channel has a total length of approximately 125 cm and has a small diameter, providing a clearance between the elevator wire and its channel of only 0.185 mm.[39] Friction resistance to the inflowing air along the wall of the elevator wire channel will reduce the air pressure along the channel. According to the Ideal Gas Law, the pressure will only increase at the lever recess, if inflow of air reaches the recess.

$$p = \frac{nRT}{V}$$

With:
p the pressure of the gas

V the volume of the gas

n the amount of substance of the gas

R the ideal gas constant

T the temperature of the gas

5.1.2. Aim

To detect malfunctioning of the O-ring seal in the elevator mechanisms of duodenoscopes, it is important that the forceps elevator lever recess is pressurized during the leakage test. The aim of this chapter is to answer the secondary research question '*What is the pressure on the O-ring seal in the elevator construction in duodenoscopes during the leakage test in the manual cleaning phase?'*.

5.2. FloXpress simulation settings

A flow analysis study was done in SolidWorks FloXpress Analysis Wizard.

Geometry

The diameter of the elevator wire was measured at 0.57 mm in an Olympus TJF-Q160. However, using a diameter of 0.38mm, a best-case scenario is created, since the elevator wire is twisted, therefore the 'center-line' is used with 2/3th of the total diameter of the wire. The radial clearance

between the elevator wire channel and the elevator wire channel has been reported to be 0.185 mm and the total length of the channel being flushed to be 125 cm. A simplified model of the elevator wire channel and its wire was made: a tube with 1,00 mm outside diameter with a length of 1250 mm within its center a solid 0.38mm-rod (*Figure 22*) with a radial clearance of 0.185mm. The ends of the models were covered by a 0.01mm-thin lid, in order to meet the solvers requirements.

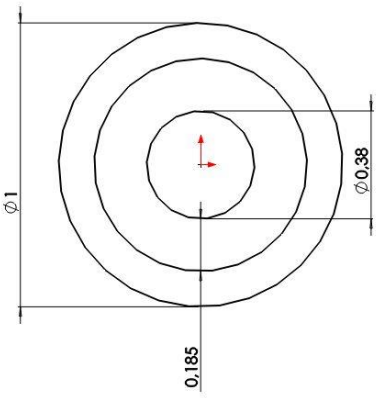

Figure 22. Input dimensions (mm) for the abstract model in SolidWorks of the elevator wire channel. This sketch is extruded for 1250 mm.

Fluid selection

The type of fluid selected was 'air'.

Model boundaries

The assumption was made that the inlet pressure at the elevator wire channel equals the inlet pressure at the leakage tester connector of 240mbar. The inlet pressure was set at 124000.00 Pa and the outlet pressure was set at 100000.00 Pa resulting in a pressure gradient along the channel of 240 mbar. The temperature was set at 293.20 K (20C°).

Solve model

A FloXpress Analysis was solved with the highest resolution.

5.3 Results of the simulation

The results of the simulation are reported in velocity [m/s] (*Figure 23*). The velocity of the air at the output of FloXpress Analysis Wizard is 0 m/s, resulting in a pressure of 0 mbar. The velocity of the airflow becomes zero after and thereby is the pressure dropped to 0 mbar, after 51% of the total length of the channel. This is at a distance of 64 cm from the inlet of the channel.

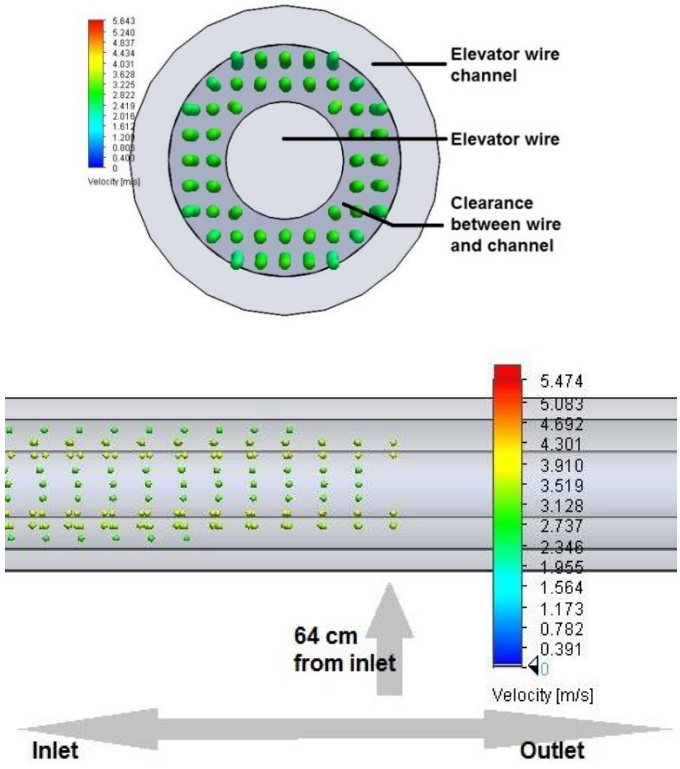

Figure 23. Top: Radial cross-section at the inlet; velocity resulting in the end of the simulation. Bottom: Longitudinal cross-section at 700mm tube length; velocity resulting in the end of the simulation.

5.4. Discussion and conclusion

'What is the pressure on the O-ring seal in the elevator construction in duodenoscopes during the leakage test in the manual cleaning phase?'.

The inflow of 240 mbar for 2 minutes into the proximal ending of the elevator wire channel was simulated with basic computational fluid dynamics analysis with a simplified model. It was concluded that the air velocity becomes 0 m/s after 70cm of the elevator wire channel; 51% of its total length. In other words, the air is not compressed after 51% of the total length of the elevator wire channel, and the elevator lever recess is not pressurized any more than the atmospheric pressure.

Since this computational model is a simplification of the real situation, a practical experiment with an Olympus TJF Q160-V (distally-unsealed model) was added for empirical enhancement. The instrument channel was sealed at the tip with wax while the distal opening of the elevator wire channel was kept free. The total distal tip was captured in an empty balloon with a tight connection (*Figure 24*). In this way, a volume change could be observed in the balloon, and thereby a pressure change. When performing the leakage test according to the manual [37] with a calibrated Olympus Mu-1 air compressor, no volume change is measured over a period of 10 minutes. As a positive control, to determine whether the balloon's stiffness is adequate for this experiment, the air compressor is directly connected to the balloons, resulting in an immediate inflation of the balloon.

Both results combined indicate that the forceps elevator lever recess is not pressurized during the leakage test, what would mean that malfunctioning of the elevator wire seal could not be detected. These findings should be considered as a pilot test; a succeeding study should be performed evaluating the effectiveness of the leakage test more profoundly. The most desirable study method would be to measure the pressure in the elevator recess with a highly sensitive pressure sensor; if the pressure during the leakage test is averaged over multiple leakage tests, then the averaged pressure will be a good indication of the pressurization of the O-ring seal in the elevator mechanism of duodenoscopes. Most preferably, the measurements are replicated using multiple duodenoscopes with a differentiation in manufacturers.

Manufacturers are strongly advised to investigate the leakage testing of the O-ring seal in the elevator mechanism of duodenoscopes in their current model duodenoscopes, and revise, if necessary, the leakage testing pathway to the distal seal, to ensure that it is pressurized to test the sealing performance. To have an effective leakage test, the connection from the inflow of air to all seals and other parts tested need to be direct and fast. A simple solution could be to create an opening in the proximal side of the forceps elevator lever recess. In this way, a direct inflow is provided from the flexible bending section which is certainly inflated during the leakage test.

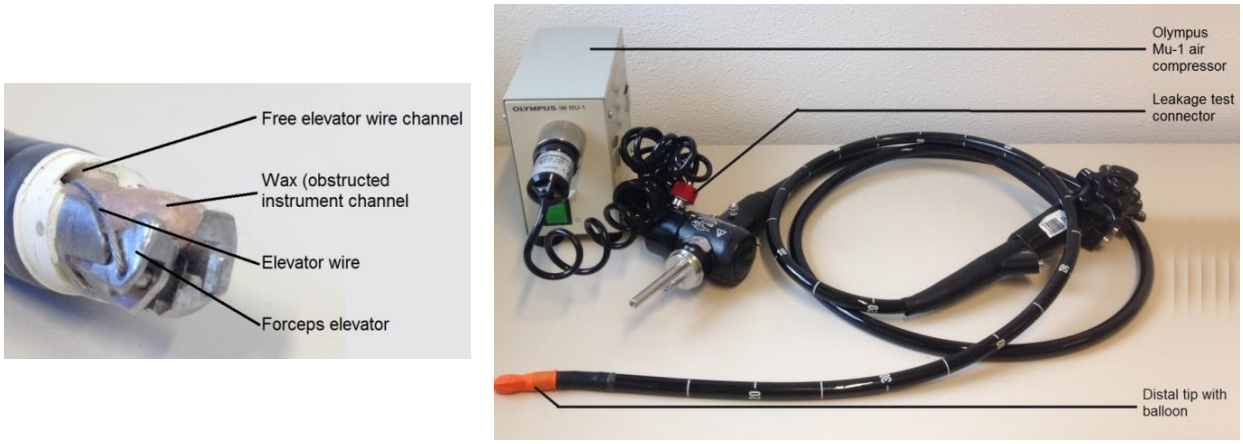

Figure 24. Testing the effectiveness of the leakage test on the distal O-ring seal. Top: the instrument channel is fully obstructed at the distal end. Bottomt: an empty balloon is placed over the distal tip. Bottom: the leakage test is performed according to the manual: no inflation of the balloon is observed.

Part C. Bacterial tightness of the distal O-ring seal in used duodenoscopes

Chapter 6. Screening of microbial contamination leaked through the seal during maintenance

6.1. Introduction

6.1.1. Routine surveillance and maintenance of flexible endoscopes

The normative guidelines on microbiological surveillance are internationally varying. [37] During routine surveillance, only the channels are checked on microorganisms. Sampling the surface of the elevator lever recess to detect leakage of the O-ring is not possible in clinical settings since the updated model duodenoscopes have a permanently fixated recess lid and a permanently fixated distal cover. The only moment to do this screening is to sample used distally O-ring-sealed model duodenoscopes if the distal tip is dismantled during maintenance. Flexible endoscopes need maintenance periodically; during maintenance, they are inspected, revised and repaired. Both manufacturers and third parties offer maintenance services.

6.1.2. Aim

The aim of this Chapter is to evaluate the sealing efficacy of the O-ring seal in the distal tip of duodenoscopes, by detecting absence or presence of microbial contamination at the clean side of the O-ring seal. The presence of microbial contamination would indicate the leakage of microorganisms through the O-ring seal. Samples of distally O-ring-sealed model duodenoscopes were taken from different surfaces in the duodenoscopes.

In this Chapter the secondary research question will be answered *'Is the clean side of the O-ring seals in duodenoscopes contaminated with microbes?*'.

Figure 25. Orange E-swab Copan

6.2. Screening method

6.2.1. Inclusion criteria

The samples were taken at endoscope maintenance center Rescope BV in Beuningen in The Netherlands. The samples were taken off all incoming duodenoscopes sent for maintenance for eight months in the period 1st of March 2016 to 1st of October 2016. The expected number of sampled duodenoscope was 32 for the distally O-ring-sealed models, based on the monthly company-average 4 distally O-ring-sealed model duodenoscopes. Distally-sealed duodenoscope models included were Olympus TJF-Q180V, Pentax ED-3490TK and Pentax ED-3670TK.

6.2.2. Sampling method

The samples were taken by swiping with an ultra-thin tipped swab over particular surfaces of interest on the distally-sealed model duodenoscope by the director of Rescope according to a strict protocol. [Attachment 2] These surfaces were the elevator recess beneath a lifted elevator, the elevator recess with the distal cover dismantled beneath a lifter elevator and the lever recess with the led dismantled. The swabs used are Orange E-Swabs of Copan Diagnostics B.V. (product number 483C) (*Figure 25*). Before dismantling the distal cap, the distal tip was disinfected with 70% ethanol. Before dismantling the elevator lever recess lid, the distal tip was disinfected with 70% ethanol again. The samples were stored in the fridge for maximally one day and transported in a sealed envelope by PostNL BV.

6.2.3. Culturing method

The samples were cultured at the department Medical Microbiology of the University Medical Center Groningen (UMCG) in The Netherlands. The swabs of the samples were immersed in a tube with

fresh BHI broth. The BHI broth tube was incubated for two days at 35°C. To check for antibiotic resistance, the positive BHI cultures were cultured again by streaking with an inoculation loop on a blood agar (BA), MacConckey number 3 agar with crystal violet (MCC3) and Sabouraud dextrose agar with aztreonam and vancomycin (SAB DEX + av) covered-petri-dishes (Media Products BV) and incubated for two days at 35°C.

6.2.4. Data interpretation

The colonies were identified using MALDI-TOF MS of Brooker BV. Sampled were considered dirty if a non-environmental or non-dermal micro-organisms is identified.

6.3. Culture results

In total three distally O-ring-sealed models were sampled; all of them were Olympus TJF-Q180V models. Only environmental microorganisms were identified in the cultures, meaning that no surfaces were found classified as dirty (*Table 12*).

6.4. Discussion and conclusion

In this experiment, bacterial leakage did not appear through the distal O-ring to lever recess. Unfortunately, only three distally-sealed model duodenoscopes were sent for repair during the study period; much lower than the expected number of 32. The most logical explanation for this lower number was the recall for distally-sealed duodenoscopes of manufacturer Olympus for the distally O-ring-sealed model duodenoscopes, consequently, these instruments were sent to Olympus B.V. for repair instead to a third party.

To obtain a robust conclusion, more dismantled distally-sealed duodenoscopes should be sampled. Most ideally, manufacturers and third party repair centers would support such a follow-up study.

Table 12. Results of surveillance of the forceps elevator mechanisms in distally-sealed model duodenoscopes

Elevator recess			**Elevator recess with dismantled distal cover**			**Lever recess with dismantled lid**		
#Sampled	#Clean	#Dirty	#Sampled	#Clean	#Dirty	#Sampled	#Clean	#Dirty
3	**3/3 (100%)**	**0/3 (0%)**	**1**	**1/1 (100%)**	**0/1 (0%)**	**1**	**1/1 (100%)**	**0/1 (0%)**

Chapter 7. Systematic investigation of the bacterial tightness of airtight seals

7.1. Introduction

7.1.1. Relevance of systematic investigation

As stated in 'Chapter 1.2. Problem definition and aim', if a distally-sealed duodenoscopes passes the leakage test, this not necessarily has to imply that the seal has sealing efficiency against micro-organisms. To gain insight into the sealing efficiency against micro-organisms, it is important to have knowledge of the sealing performance of instruments' seals during use.

In clinical settings, the distal O-ring seal endures various factors that could –on theoretical basis- influence the sealing efficiency against micro-organisms. For example, the distal O- is a quasi-dynamic seal, installed in the dynamic rotational moving axle of the elevator mechanism. Manipulation of the lever axle results in movements of the O-ring, possibly creating space for microbes to pass along the sealing surface. Likewise, the seal endures pressure resulting from fluid flow during reprocessing and resulting from intra-abdominal pressure during ERCP procedures. The pressure resulting from fluid flow during reprocessing can be induced during manual cleaning by the syringe or during automatic cleaning by the rinsing water of the AER machine. This pressure gradient along the seal theoretically could promote bacterial leakage. Besides, there are more factors that vary during use that may have influence on microbial leakage through the seal, in this Chapter referred as 'usage variables';

- Condition of the O-ring;
- Density and viscosity of the medium at the dirty side of the O-ring;
- Frequency of rotation of the axle;
- Speed of rotation of the axle;
- Type of microorganism(s);
- Environment temperature.

Next to these factors, other factors can be introduced by variations in the design and manufacturing process, in this Chapter referred as 'product variables'. The product variables are discussed in 'Chapter 4.1.2. O-rings' sealing performance'.

7.1.2. Microbial leakage evaluation methods

To evaluate the bacterial sealing efficiency in a systematical manner, leakage of bacteria need to be identified and ideally quantified. In the research field of dental implant, leakage of microorganisms is evaluated since 1861 (Chapter Chapter 2. Reported-infection safety adverse events related to O-ring seals in medical instruments). Resulting, many methods are explored for the evaluation of microbial leakage. They are briefly introduced below, including a short discussion of their advantages and disadvantages. [56]

Air pressure

Using air pressure, one side of the seal is pressurized by compressed air. Both examination of the release of air bubbles at the shielded-side and marginal pressure reduction in indicates air leakage. Air pressure studies only provide information about the existence of a leakage path for air.

Dye penetration

For evaluating microbial leakage for dental implants, the most popular technique makes used of dye penetration with colored agents. This method allows visual examination of the leakage path, but is highly sensitive for the particle size of the dye, and is not necessarily representative for microbial behavior.

Chemical tracers

Chemical tracers are non-radioactive, and these studies rely on a chemical reaction between one or more chemicals indicating leakage. The results of chemical tracers are sensitive for interpretation; therefore, its quality highly depends on the chosen tracer(s). Moreover, this technique is highly sensitive for the particle size of the dye, and is not necessarily representative for microbial behavior.

Bacterial penetration

Bacterial penetration studies indicate leakage of bacterial along the sealed interface. These studies cannot show leakage of smaller microorganisms than bacteria, for example of viruses and spores. Because in vivo studies bacterial studies require artificial media, in vitro studies are more clinically relevant. The studies interpret their data rather qualitative than quantitative, due to the difficulty in controlling the bacterial population.

Scanning electron microscopy

Macroscopic analysis of the sealed interface can provide information about the behavior of the seal and leakage patterns; microorganisms could be visualized along this interface. A small field of view is the most important disadvantage of this type of study.

Radioisotope studies

Radioactive isotopes could be used to study radioisotope leakage over the sealed interface: leaked radioisotopes can be detected with the use of radiography. The results are sensitive to standardization (exposure of the radioisotopes to the radiograph and type of radioisotope) and assessment (translating the resolution of the radiograph to leakage rates). Moreover, this technique is highly sensitive for the particle size of the isotope, and is not necessarily representative for microbial behavior.

7.1.3. Aim

The aim of this study is to determine whether O-ring seals that are designed and manufactured according to the ISO-standards have sealing efficiency against micro-organisms by evaluating the microbial tightness while the O-ring seal in the distal tip of duodenoscopes is used as a case study. It is most clinically relevant to do controlled experiments on real instruments. However, it is impossible to control the experiment conditions precisely on actual duodenoscopes; therefore, mockups of the O-ring sealing construction imitating the elevator mechanism are used. A systematic investigation of the bacterial tightness - controlling all factors that may influence microbial leakage- will provide detailed insight of the sealing efficiency against micro-organisms of O-ring seals.

Bacteria are the micro-organisms with the largest size: for bacterial leakage, marginal gaps are needed of minimally 0.5µm-1.0µm and viruses, molecules, bacterial products and ions are even smaller.[56] If bacterial leakage is found, leakage of bacterial products, viruses, molecules and ions is expected too. Since this study specifically studies the leakage of bacteria through the seal, the study method 'bacterial penetration' is considered as most clinically relevant. The bacterium used for this bacterial penetration study is *K. Pneumonia*, since this bacterium is known causing outbreaks associated with duodenoscopes in clinical settings.

In this Chapter, the secondary research question will be answered *'Are airtight distal O-rings of Olympus TJF-Q180V duodenoscopes also tight for bacteria?*' This is done using the following tertiary research questions:

- *'How should the product variables need to be controlled during the systematic investigation such that mockups reproduce the distal O-ring sealing construction in duodenoscopes?'*
- *'Are the mockups a valid reproduction of the elevator construction of Olympus TJF-Q180V duodenoscopes?'*
- *'How should the usage variables need to be controlled during the systematic investigation such that a test run mimics usage of the elevator mechanisms of duodenoscopes?'*

- *'What are the influences of the product variables on the air- and bacterial tightness of seals (e.g. mockups that are passing the leakage test) with varying usage variables?'*
- *'What are the influences of the usage variables on the bacterial tightness of airtight seals (e.g. mockups that are passing the leakage test)?'*

7.2. Methods of the systematic investigation

7.2.1. Approach

Choosing the independent variables

The variables that need to be controlled in the systematic investigation are the variables that possibly can influence microbial leakage (*Table 14*). Based on the variables, the experiment of the systematic investigation is designed; the variables expected to influence microbial leakage the most are chosen as independent variable. The independent usage variables are categorized in levels; one level is set at the minimal value (in other words zero) and the other level is set at the maximal value. The usage variables chosen as independent variable are: pressure at the dirty side, the frequency of the axle and axial displacement of the axle (*Table 15*).

- **The pressure at the dirty side** can be created during ERCP procedures inside the patient's body or during reprocessing resulting from the flowing cleaning and disinfection fluids. Pressure induced by fluid flow during reprocessing on the O-ring seal is not yet defined, and therefore not studied. Pressure on the O-ring by automatic cleaning by the rinsing water of the AER machine (maximal 5bar in the channels) is expected to approach zero and therefore has a negligible effect on the pressure at the dirty side.[57] The intra-abdominal pressure during ERCP procedures is reported in bovine animals to be 16 mbar above atmospheric pressure.[58] This value for the pressure is used to simulate the intra-abdominal pressure on the dirty side of the seal during the experiment.
- **The frequency of use** of the forceps elevator axle is also chosen as an independent variable. The frequency of use of the forceps elevator was not found in literature. The frequency of use is estimated based upon the maximal exchanges of instruments in the instrument channel of 6.[59] It is assumed that with each exchange of instruments, the forceps elevator is used 5 times, resulting in a total use of the forceps elevator of 30 times per ERCP procedure.
- **Axial displacement** is expected to have an effect on microbial leakage; due to clearance in the construction, the elevator and the elevator lever can have a slight axial displacement, which may lead to a rolling O-ring in its housing with as effect bacteria transportation along the seal. By observation of the forceps elevator of an Olympus TJF-Q180V duodenoscope, the maximal axial displacement is estimated on 0.375mm.

The product variable chosen as an independent variable is the effective compression of the installed O-ring. Of all usage variables, the effective compression affects the sealing surface the most, and thereby the ease of bacterial leakage; a tighter seal has a larger sealing surface and thereby is expected to have less bacterial leakage. The minimum and maximum effective compression ranges that are use are those found in the literature search in 'Chapter 4.2.2. Search for standardized dimensions recommended in literature'. The effective compression of the O-ring seals is categorized in two levels; low effective compression and high effective compression advised in literature.

Table 13. Independent product variables

Independent product variable	Input measure	Minimal	Maximal	Comparison to duodenoscopes
Effective compression of the installed O-ring	Percentage	Low [0-15%]	High [15-30%]	Empirical: lower and upper boundaries of the measured effective compression ranges [50], [55]

Table 14. Variables that are expected to influence microbial leakage

Product variables	Usage variables
• Bearing length • Radial clearance • Effective compression of the installed O-ring • Hardness of the O-ring • Material of the housing • Material of the O-ring • Cross-section of the O-ring (sealing contact surface) • Stretch of the installed O-ring • Surface finish of the housing at the seal-contact-surface • Surface finish of the O-ring	• Angel of rotation of the axle • Angular displacement of the axle • Axial displacement of the axle • Condition of the O-ring (aging) • Density and viscosity of the medium at the dirty side • Frequency of rotation of the axle • Pressure at the dirty side • Speed of rotation of the axle • Type of microorganism(s) • Environmental temperature

Table 15. Independent usage variables

Independent usage variable	Input measure	Minimal	Maximal	Comparison to ERCP
Axial displacement of the axle	Millimeter	No displacement [0 mm]	Displacement [0.375 mm]	Empirical: maximal axial displacement of the forceps elevator measured in an Olympus TJF-Q180V
Frequency of the axle	Number of full elevator movements per run	Passive [0 per run]	Maximum use [30 per run]	Empirical and literature: maximum use elevator based on instrument exchanges [59], [Attachment 4]
Pressure at the dirty side	Millibar	No pressure [0 mbar]	Maximum pressure [16 mbar]	Literature: Maximal intra-abdominal pressure [58]

Determining the constant variables

The variables that are not chosen as independent variables are kept constant during the controlled investigation. The values of the usage and product variables kept constant are listed and the motivated; they are chosen optimal for improving sealing efficacy, or chosen as measured as in the Olympus TJF Q180V (*Table 16.*).

Choosing the dependent variables

Two dependent variables are chosen for this study:

- To be able to measure bacterial leakage, **bacterial penetration** at the sealed-clean side must be measured. Or in other words, bacteria presence must be indicated. Bacteria presence can be indicated using the ATP level to indicate the level of protein presence and the number of colony forming units (CFU) can be counted or the turbidity of the sample can be observed after incubation of the sample to indicate whether bacteria are present. Turbidity of the sample indicates presence of bacteria; a clear sample indicates no presence of bacteria. Preferably bacterial penetration is quantified to have insight in the amount of leakage. Additionally, it is desirable that determination of the bacteria species is possible, to detect if the bacteria indicated are indeed leaked through the seal, and are not environmental contamination, since this reduces the need for a full sterile method. Finally, it would be convenient if the bacterial penetration can directly be measured after sampling, to reduce experimental time. The culturing methods are evaluated on these important requirements to be able to select the dependent variable (*Table 17-top*). Measuring bacterial penetration in the number of CFU has the advantage that bacteria can be quantified and the CFU can be individually identified. The first dependent variable is the

number of CFU; the culturing methods with the highest score. Presence of CFU indicates bacterial penetration, and absence of CFU indicates bacterial tightness.

- The second dependent variable is the **seals' airtightness**. This is evaluated using the same leakage test as clinically used; the seal is considered air penetration if pressure drops, and air tight if pressure remains on the same level.

Table 16. Constant usage and product variables

Constant usage variable	Value	In comparison to ERCP
Maximum angle of rotation of the axle	90°	Empirical: maximum rotation of the elevator measured in an Olympus TJF-Q180V
Immersion time of bacteria suspension at the dirty side	1 hour	Literature: maximum duration of an ERCP procedure [59]
Angular displacement of the axle	0mm	Optimal
Bearing length of the construction	0.65 mm	Observed: measured in an Olympus TJF-Q180V (Chapter 4.3.1.)
Condition of the O-ring (aging)	New, sterilized	Optimal
Density of the medium at the dirty side	Density of bacteria suspension ($0.96*10^3$ kg/m^3)	Standard
Duration of a full elevator movement (90°-movement)	1 second	Calculated: based on estimation of speed of manual adjustment of the elevator
Viscosity of the medium at the dirty side	Density BHI broth (undefined)	Standard, but undefined
Environmental temperature	Controlled lab temperature of 21°C	Standard
Type of microorganisms	Klebsiella Pneumonia	Literature: bacteria most frequently associated with MDRO outbreaks [23], [60]–[63]

Constant product variable	Value	In comparison to duodenoscope
Bearing length of the construction	0,65	Observed: measured in an Olympus TJF-Q180V (Chapter 4.3.1.)
Radial clearance	0.15mm	Observed: measured in an Olympus TJF-Q180V (Chapter 4.3.1.)
Hardness of the O-ring material	Durometer 70 shore A	Literature: optimal hardness for rotary seals [50], [51], [54], [55]
Material type of the construction	Stainless steel	Observed: material of an Olympus TJF-Q180V (Chapter 4.3.1.)
Material type of the O-ring	Silicone	Empirical: estimated material type based on observation of the O-rings in duodenoscopes of Olympus
Size of the O-ring (sealing contact surface)	Approximately 2.00x0.500	Observed: measured in an Olympus TJF-Q180V (Chapter 4.3.1.)
Stretch of the installed O-ring material	1-4%	Literature: optimal stretch range [50], [51], [54], [55]
Surface finish of the construction at the seal contact surface	Smooth	Literature: smooth surface finish required for surfaces of the O-ring housing in contact with O-ring [50], [51], [54], [55]
Surface finish of the O-ring	Standard of manufacturer	Standard, undefined

Table 17. Weighting methods on important requirements for a systematic investigation. Top: Sampling method. Bottom: Culturing method.

Sampling method		Swab		BHI broth with direct immersion		BHI broth with indirect immersion	
Requirement	Weight	Score	Weighted score	Score	Weighted score	Score	Weighted score
High sampling-density of surfaces	3	1	3	3	9	2	6
Possibility for spatial leakage measurement	1	2	2	3	3	1	1
Low possibility of influencing bacterial penetration	4	2	8	1	4	3	12
Total scoring for requirement fulfillment			**13**	**16**		**19**	

Culturing method		ATP level		#CFU in broth		Turbidity of broth	
Requirement	Weight	Score	Weighted score	Score	Weighted score	Score	Weighted score
Directly measure bacterial penetration	1	1	1	3	3	3	3
Determination of bacteria possible (i.g. non-sterile methods required)	4	1	4	3	12	2	8
Bacterial penetration quantification	3	3	9	3	9	1	3
Total scoring for requirement fulfillment			**14**	**24**		**14**	

Choosing the sampling method

To be able to measure bacterial penetration, leaked bacteria must be sampled from the clean side. Possible sample methods are a swab, direct immersion with BHI broth and indirect immersion with broth. With sampling with a sterile swab, the clean side is swabbed wiping all surfaces. With sampling with direct immersion, BHI broth is inserted in the clean basin at the beginning of the experiment and at the end removed after. With sampling with indirect immersion, BHI broth is inserted in the clean basin at the end of the experiment and directly removed after refluxing the broth. The sampling method for this experiment must have a high assurance of sampling the total surface of the clean side. Additionally, it could be convenient to be able to discriminate between contaminated surfaces, to be able to measure the depth of bacterial penetration. Also, the amount of bacterial penetration over time could give valuable information about the bacterial penetration behavior. Finally, it is important that the sampling method does not influence the bacterial penetration and thereby biases the measured effect. The requirements for the bacterial penetration methods were scored and weighted on the importance (*Table 17-bottom*). Since indirect immersion with BHI broth is the sampling method with the highest score, this method was used to sample bacteria at the clean basin to indicate bacterial penetration.

Design of the set-up construction

The manufacturing method used as high precision mechanics since assurance of obtaining close tolerances is needed. To optimize the design, a list of requirements was made of all factors influencing the validity and reliability of the set-up construction. The most requirements were:

- It must be possible to closely **contro**l all the **independent variables**.
- The construction of the mockups must be a **faithful replication** of the seal construction in the tip in distally-sealed model duodenoscopes. Therefore, is must be possible to manufacture

the mockups with close tolerances and thereby a low tolerance stack-up and the connection of axles in the construction must be possible to manufacture with a high concentricity.

- The setup must be **easy to manufacture** and parts
- The construction must be **reproducible**. To decrease the effects of deviations in manufacturing, the mockups were duplicated to a maximum number suitable for the budget. The budget was €500,- provided by the department Medical Microbiology UMCG.
-
- The construction of the mockups must **reduce** the chance on **cross-infection** between basins, it must be possible to possible clean, disinfect and sterilize the construction and the construction of the mockups should enhance sterility assurance during sampling by a low number of required steps to do sampling.
- It must be possible to perform a **leakage test**.
- The construction must be easy to **(dis)assemble and sample**.

7.2.2. Set-up

The set-up consisted of three aspects: the experiment construction, bacteria suspension, leakage tester and disinfection and sterilization equipment. All aspects are explained in detail in the next paragraphs.

7.2.2.1. Construction

The construction was built from of several parts; a trifold parallel mockup, installation assistance parts, support assistance parts, parts for preventing contamination, parts for motion simulation, parts for pressurization simulation (*Figure 26*). The independent usage variables that needed to be controlled are the frequency of dynamic rotational loading and the pressure on the dirty side. To simulate the usage variables, the parts for motion simulation and parts for pressurization simulation could be used.

Trifold parallel mockup

A trifold parallel mockup construction was made from 3 stainless steel blocks with a clean and dirty basin, 3 stainless steel axles and two sizes of O-rings, in order to replicate duodenoscopes' distal sealing construction (*Figure 28-top*). For assembly of the axles, a hex key was used.

The stainless steel blocks and axles were designed in accordance with standars advices by manufacturers (since ISO 3601-2 cannot be apply to constructions at this scale) with the dimensions the elevator mechanism as measured in the Olympus TJF-Q180V. [Attachment 10] (*Table 18, Figure 27*). The axles and blocks were manufactured in the Research Instrument Workshop of the UMCG using construction drawings with close tolerances: for the dimensions influencing defining the sealing efficacy tolerance ±0.01 mm is used, and for the other dimensions the tolerance ±0.05 mm is used. Measurement of the dimensions that possible could influence leakage was done using optical measurement with 0.01mm accuracy (Philips Coordinate Drill Press), a micrometer with 0.01 mm accuracy (Mitutoya external 0-25mm) or a caliper with 0.02 mm accuracy (Mohr Digital 16EWR) (*Table 18, Figure 27, Table 20*). [Attachment 5]For use of O-rings, silicone O-rings were searched in the O-ring manufacturers product databases with sizes such that each would fit in the mockup construction with the effective compression ranges (one size for lowly-compressed seals and one size for highly-compressed seals) while keeping the constant variable 'inside diameter stretch range' in the range of 0-4%. Silicone O-rings are used of the sizes 2.31x0.46mm and 2.31x0.56mm (Apple Rubber Products BV, compound designation: SL). When installed in the designed set-up [Attachment 6], the size 2.31x0.46mm results in an effective compression inside 6-15% and the size 2.31x0.56mm results in an effective compression of 23-30%. With correction for deviating dimensions due to fabrication tolerance, the effective compression of the O-ring installed in the actual set-up is for the lowly-compressed seals 0-13% and for the highly-compressed seals 13-28% (*Figure 27, Table 18*). The dimensions of the mockups were similar to those of the elevator mechanism, with exception of the radial clearance (higher in the mockups), the bearing length (larger in the mockups) and the stretch (lower in the mockups).

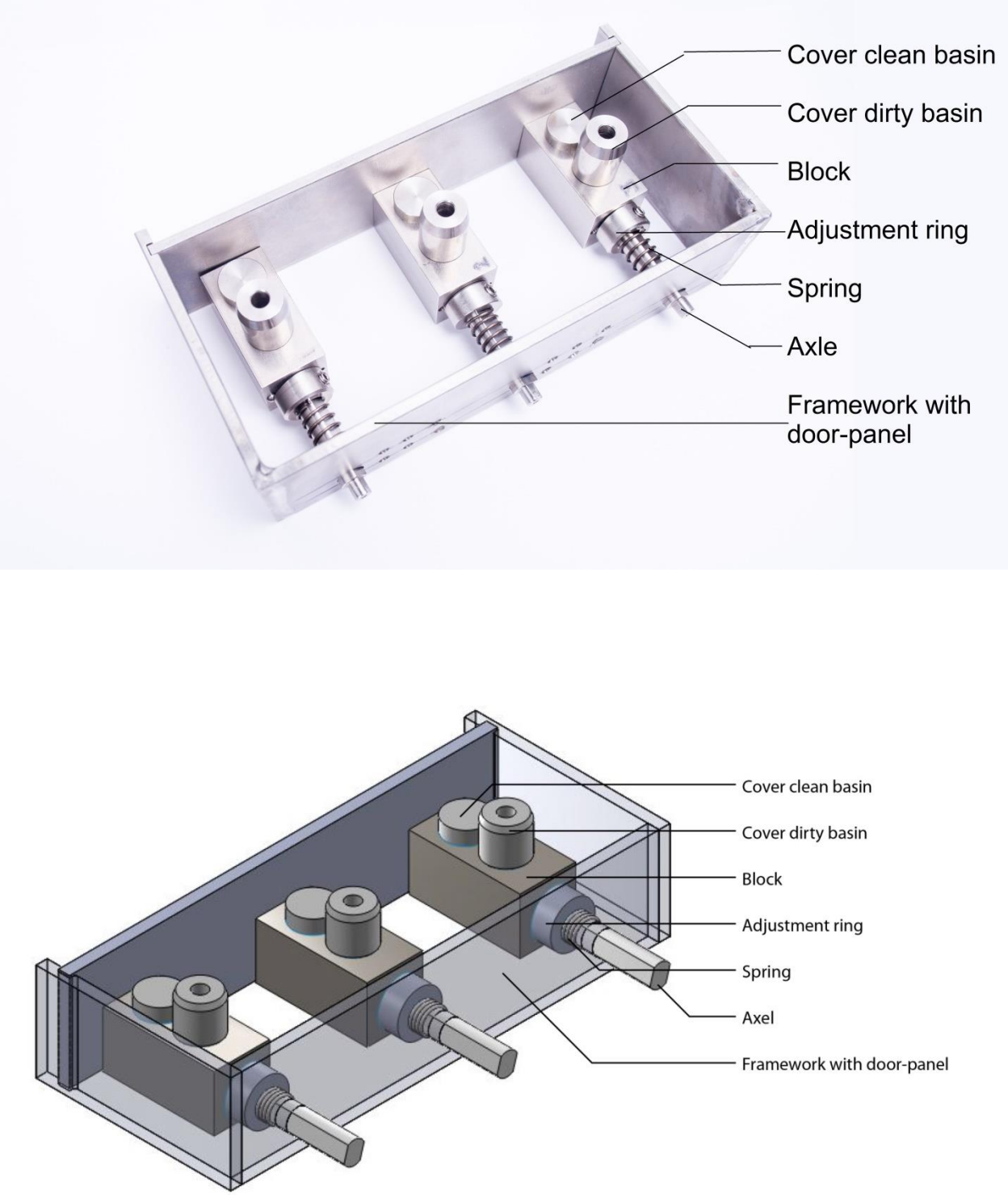

Figure 26. Experiment construction: a trifold parallel mockup, installation-assistance parts, parts for preventing contamination, parts for motion simulation, parts for pressurization simulation. Top; picture of the actual construction. Bottom: abstract representation of the construction.

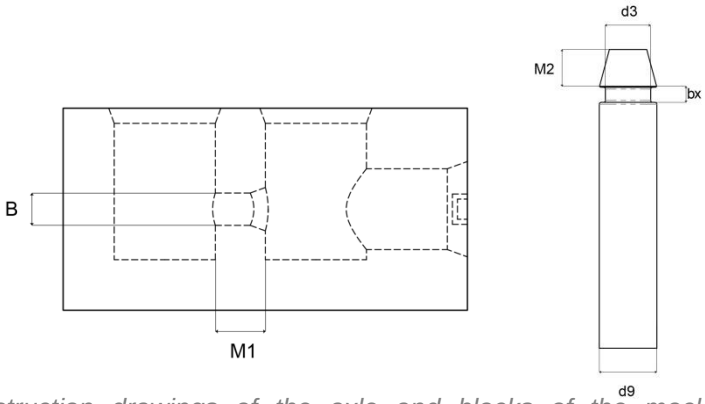

Figure 27. Construction drawings of the axle and blocks of the mockups including labels of dimensions directly measureable. Left: block. Right: axle.[Attachment 10]

Table 18. Stretch and effective compression measured in the Olympus TJF-Q180V and in the mockups. (min=minimum dimension with correction of tolerances and measurement accuracies and max=maximum dimension with correction of tolerances and measurement accuracies) (=directly measurable in the construction and **= calculated using formulae of Table 19)*

Dimension	Symbol	Measured in Olympus TJF-Q180V	Design for mockups with a lowly-compressed seal	Actual mockups with lowly-compressed seal	Design for mockups with highly-compressed seal	Actual mockups with highly-compressed seal
Inner diameter of O-ring* (mm)	$d1_{min}$	2,07	2,30	2,30	2,30	2,30
	$d2_{max}$	2,13	2,32	2,32	2,32	2,32
Cross section of O-ring* (mm)	$d1_{min}$	0,47	0,45	0,45	0,55	0,55
	$d2_{max}$	0,53	0,47	0,47	0,57	0,57
Piston diameter*(mm)	$d9_{min}$	3,02	3,04	2,96	3,04	2,96
	$d9_{max}$	3,08	3,06	2,99	3,06	2,99
Bore diameter* (mm)	$d4_{min}$	3,17	3,19	3,19	3,19	3,19
	$d4_{max}$	3,23	3,21	3,21	3,21	3,21
Housing inside diameter * (mm)	$d3_{min}$	2,37	2,39	2,28	2,39	2,28
	$d3_{max}$	2,43	2,41	2,37	2,41	2,37
Radial housing depth** (mm)	t_{min}	0,37	0,39	0,41	0,39	0,41
	t_{max}	0,43	0,41	0,47	0,41	0,47
Diametrical stretch inside diameter O-ring installed** (mm)	s_{min}	0,24	0,07	0,00	0,07	0,00
	s_{max}	0,36	0,11	0,07	0,11	0,07
Percentage of inside diameter stretch**	S_{min}	11	3	0	3	0
	S_{max}	17	5	3	5	3
Percentage of O-ring cross-sectional reduction resulting from diametric stretch** (%)	R_{min}	6,62	2,30	0,00	2,30	0,00
	R_{max}	9,22	2,79	2,30	2,79	2,30
Cross section installed O-ring** (%)	$d2_{min}$*	0,43	0,44	0,44	0,53	0,54
	$d2_{max}$	0,49	0,46	0,47	0,56	0,57
Percentage of effective O-ring cross-section compression** (%)	C_{min}	0	6	0	23	13
	C_{max}	25	15	13	30	28

Table 19. Formulae used to calculate dimensions not directly measurable

Dimension	Symbol	Formula [1]
Radial clearance	g_{min}	$= (d4_{min} - d9_{max})/2$
	g_{max}	$= (d4_{max} - d9_{min})/2$
Radial housing depth	t_{min}	$=(d4_{min} - d3_{max})/2$
	t_{max}	$=(d4_{max} - d3_{min})/2$
Diametrical stretch inside diameter O-ring installed (mm)	s_{min}	$=d3_{min} - d1_{max}$
	s_{max}	$=d3_{max} - d1_{min}$
Percentage of inside diameter stretch	S_{min}	$=(s_{min}/d1_{max})*100[\%]$
	S_{max}	$=(s_{max}/d1_{min})*100[\%]$
Percentage of O-ring cross-sectional reduction resulting from diametric stretch	R_{min}	$= 0,01 + 1,06(S_{min}) - 0,1 (S_{min})^2$
	R_{max}	$= 0,01 + 1,06(S_{max}) - 0,1 (S_{max})^2$
Cross section installed O-ring (mm)	$d2^*_{min}$	$=d2_{min} - d2_{min}(R_{max}/100)$
	$d2^*_{max}$	$=d2_{max} - d2_{max}(R_{min}/100)$
Percentage of effective O-ring cross-section compression	C_{min}	$=(d2^*_{min} - t_{max})/d2^*_{min}*100[\%]$
	C_{max}	$=(d2^*_{max} - t_{min})/d2^*_{max}*100[\%]$
Bearing length	M_{min}	$=M1_{min} - z1_{max} - z2_{max} - dx_{max}$
	M_{max}	$=M1_{max} - z1_{min} - z2_{min} - dx_{min}$

Table 20. Dimensions of the three mockups including tolerances and measurement accuracies (i=optical measurement, ii=micrometer and iii=caliper) (=directly measurable in the construction and **= calculated using formulae of Table 19) [Attachment 5]*

Dimension	Symbol	Dimension measured in Olympus TJF-Q180V ± measurement accuracy	Dimension intended in set-up ± tolerance	Dimension measured in set-up ± measurement accuracy
Piston diameter*(mm)	d9	3,05±0,03	3,05±0,01	[2,97-2,98]±0,01ii
Bore diameter* (mm)	d4	3,20±0,03	3,20±0,01	[3,20-3,20] ±0,01i
Housing inside diameter* (mm)	d3	2,40±0,03	2,40±0,01	[2,29-2,36] ±0,01i
Width of the O-ring housing*	bx	0,80±0,03	0,80±0,01	[0,79-0,80] ±0,02iii
Bearing length of the housing* (mm)	M1	Not measured	4,95±0,01	[4,95-4,96] ±0,02iii
Length of the housing's lead-in chamfer * (mm)	z1	Not measured	1,50±0,01	[0,64-0,82] ± 0,02iii
Length of the axle' s lead-in chamfer * (mm)	z2	Not measured	2,00±0,01	[2,00-2,01]±0,02 iii
Bearing length** (mm)	M	0,65±0,03	0,65±0,03	[1,33-1,52] ±0,06
Radial clearance** (mm)	g	0,075±0,06	0,075±0,02	[0,11-0,12]±0,04

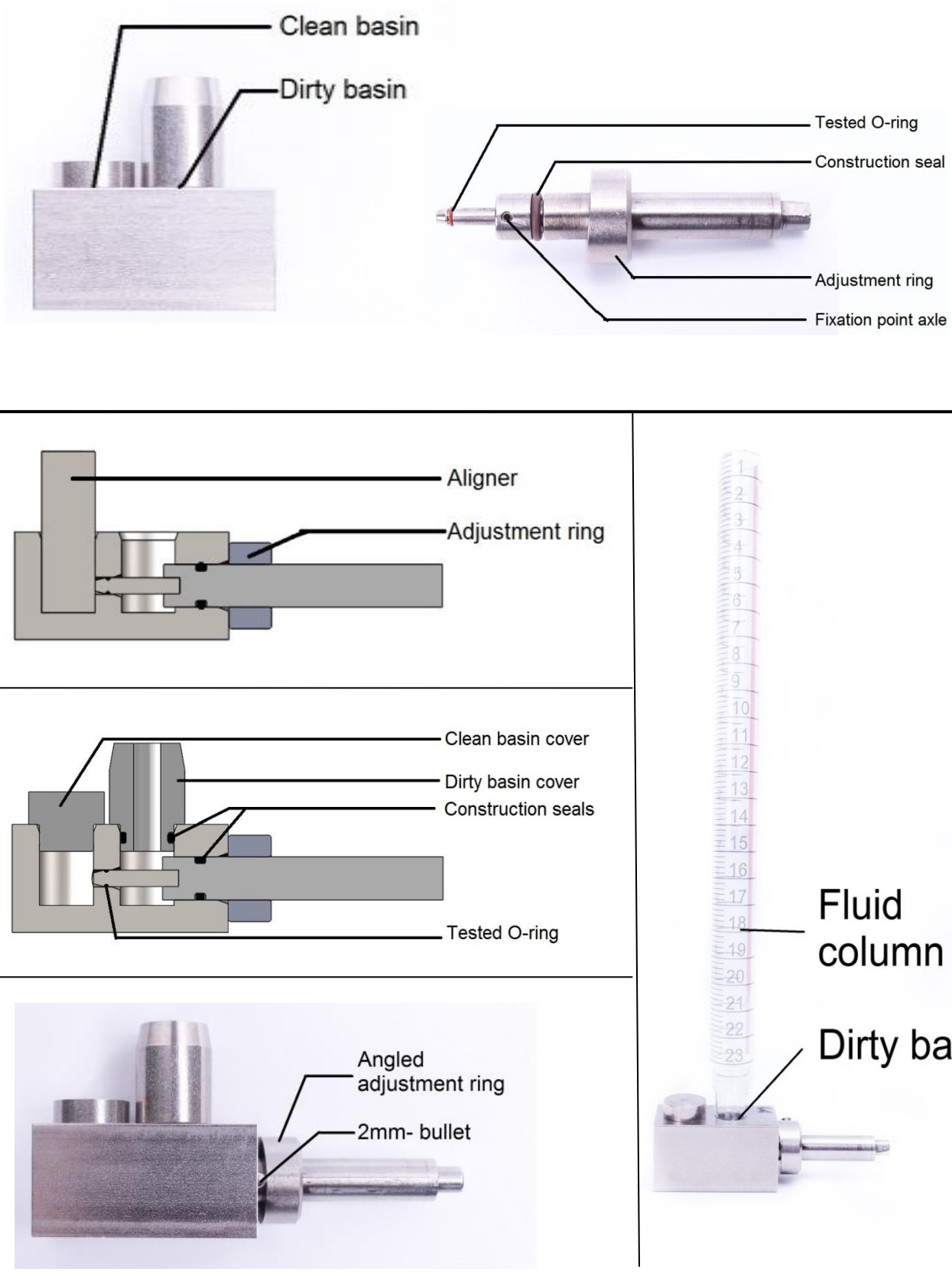

Figure 28. Pictures and drawings of mockups. Top: picture of block and axle. Right: parts for pressurization simulation. Left-top: installation assistance parts. Top-middle: parts for preventing cross-contamination. Left-bottom: parts for motion simulation.

Installation assistance parts

Installation assistance parts were used to position the mockup axles in the mockup blocks (*Figure 28-left-top*). The installation assistance parts are one stainless steel aligner and three adjustment rings. The aligner and three adjustment rings were manufactured in the Research Instrument Workshop of the UMCG from stainless steel using construction drawings. [Attachment 10]

The distal end of the mockup axles must be aligned with the outside of the clean side basin. With the use of an aligner fitting in the clean basin [Attachment 10], the axles were assembled in this manner. The axles are fixated in this position fastening adjustment rings at the mockup axle outside the basin block. For the fixation of the adjustment rings, a stainless-steel hex key was used.

Support assistance parts

Support assistance parts were used to fixate the mockup axles and –blocks. The support assistance parts were a stainless-steel framework with a sliding door panel and three stainless steel springs (*Figure 27*).

The stainless-steel framework was manufactured in the Research Instrument Workshop of the UMCG using construction drawings. [Attachment 10] The springs were used to keep the axles in the correct position in the blocks.

Parts for preventing contamination

Both basins of the mockup blocks must not be contaminated by the environment, or must not cross-contaminate each other. To prevent this, covers were made for both basins. The cover of the dirty basin had and a cylindrical opening, since pressure must be applied on the dirty side (*Figure 28-left-middle*).

Parts for motion simulation

Rotational loading of the axles was done by manual control. Each axle was turned 90° back-and-forth for 30 times over a total period of 60 minutes by repeating the same cycle every two minutes; a 1 second movement of 90° forth, was followed by a one minute break, followed by a 90° backwards one second movement. The rotational loading of the axle was bare by the framework, springs and adjustment rings.

To facilitate translational motion of the axles, a sliding system was used; three stainless steel angled adjustment rings and 3 stainless steel 3-mm bullet. The three angled adjustment rings were manufactured in the Research Instrument Workshop of the UMCG from stainless steel using construction drawings. [Attachment 10] If the translational motion of the axle as required, the angled adjustment rings are used instead of the non-angled adjustment rings and the 3-mm bullet is installed in the 3-mm diameter holes in the blocks with 1.5 mm depth. Once installed, 90° rotation of the axle results in translational displacement of the mockup axles; the 3-mm bullet pushes the angled surface of the adjustment ring and thereby, axially displaces the axle for 0.375mm (*Figure 28-left-bottom*).

Parts for pressurization simulation

Fluid columns were used to simulate gauge pressure on the dirty side of the elevator lever system. The fluid column tube was made of a 25-mL sterile pipette (Greiner CELLSTAR® serological pipette) with the narrow tip and the end cut off (to lower the chance of capturing of air bubbles during filling of the fluid column) (*Figure 28-right*). The tube is sealed in the basin of the dirty side using Orin wax. The height of the pressure column is calculated using the hydrostatic formula.

$$p = p_0 + \rho * g * h$$

Where:

p = pressure on point of interest

p_0 = atmospheric pressure on the fluid surface

ρ = density of the fluid (kg /m^3)

g = gravitation constant (m/s^2)

h= height of fluid above point of interest (m)

The desired gauge pressure in the construction on the height of the O-ring was 16 mbar (=16*10^2 Pa). The density of the full growth bacteria suspensions was calculated using the mass and volume. A volume of 5,00 mL was weighed on a Mettler AB54-S precision scale (±0.1 mg). The mass of 5.00 mL is 4,880*10^{-3} kg. The density of the full-growth bacteria suspension was calculated to be 0.96*10^3 kg/m^3. Therefore, columns need to be filled up to the 5mL scale in the Greiner tube to have a total fluid column of 16.7 cm. It is important to fill the fluid column slowly, to prevent the capture of air bubbles in the fluid.

$$\rho = \frac{m}{V}$$

Where:
m = mass (kg)

V=volume (m^3)

$$\rho = \frac{m}{V} = \frac{4{,}880 * 10^{-3}}{5{,}00 * 10^{-6}} = 0{,}976 * 10^3\ kg/m^3$$

$$h = \frac{p}{\rho * g} = \frac{16 * 10^2}{0{,}976 * 10^3 * 9{,}91} = 0{,}167\ m$$

7.2.2.2. Bacteria suspension

Full-growth BHI broth with a fully antibiotic-sensitive *K. Pneumonia* strain (ATCC 13883) was used. A sterile tube with 10 mL BHI broth was inoculated by one CFU of the strain. The inoculated BHI broth was cultured to a full-growth suspension in an incubator at 35°C overnight. K. Pneumonia suspension was stored in the fridge at 9°C for maximally 3 days.

7.2.2.3. Leakage tester

To test the seals' airtightness, manual sphygmomanometer (Heine Gamma XL) with a range of 0-300 mmHg gauge was refurbished (*Figure 29-left*); the machete and the handcuff were removed the meter, and with a T-connection 6x6x6mm (Vijvercompleet.nl) the handcuff was reconnected to the dial. To connect the outlet of the T-conection to the dirty side cover, a 13mm PCV 40cm-tube (Vijvercompleet.nl) was attached using a straight connector 6x13mm (Vijvercompleet.nl). The leakage tests were performed by pressurizing the dirty side basin to 180 mmHg (≈240 mbar) (*Figure 29-right*).

7.2.2.4. Disinfection and sterilization equipment

The silicone O-rings were sterilized in a fine-meshed stainless steel container (Spice ball, Professional Line 9cmØ) using steam sterilization (Sanamij B.V.) at 121±1°C for 50 minutes, and after directly inserted per three into a separate sterile cup with sterile tweezers. They were stored for maximally 5 days.

In between the experiment runs the construction were washed, disinfected and sterilized. The construction parts were washed and disinfected after each run: two times by washing with 800µL demineralized water, followed by disinfecting with 800µL 70% ethanol. After washing and disinfection, the construction parts were sterilized using steam sterilization (Sanamij B.V.) at 121±1°C for 15 minutes.

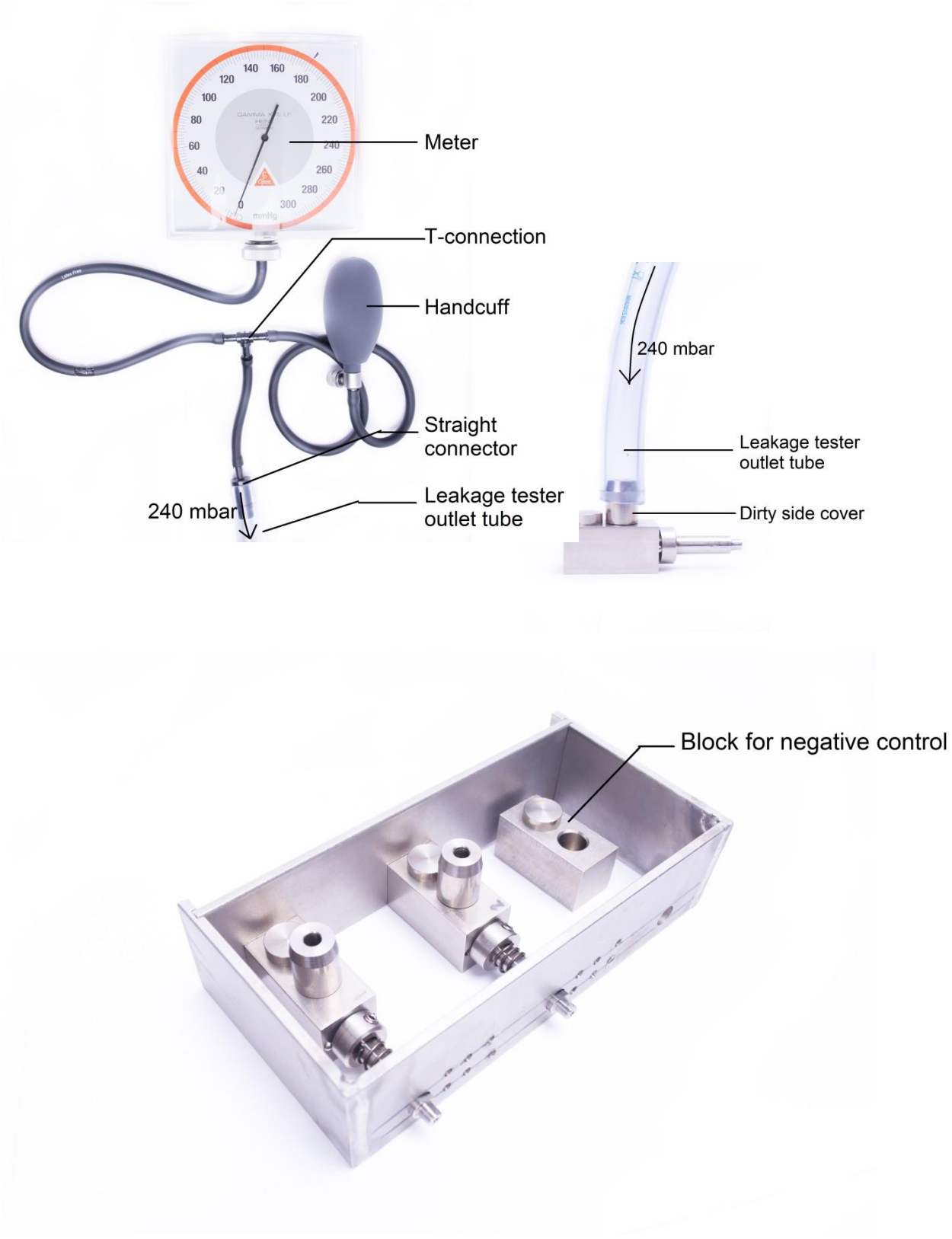

Figure 29. Additional equipment for setup. Top-left: the outlet of the leakage tester can be connected to the dirty side cover. Top-right: the refurbished leakage tester. Bottom: contruction with a blcok for the negative control group.

7.2.3. Systematic investigation design

7.2.3.1. Independent and dependent parameters

The independent variables for the systematic investigation of the seal tightness were the gauge pressure at the dirty side, the frequency of the dynamic rotational loading, the axial displacement and the effective compression of the installed O-ring. All four independent variables were categorized into two levels.

The dependent parameters were air and bacterial tightness; respectively the results of the leakage test and the results of the cultures. The two dependent parameters were also categorized in two levels. Results of the leakage test were categorized in the levels 'air tight' or 'air penetration', and results of the cultures were categorized in the levels 'bacterial tight' or 'bacterial penetration'.

7.2.3.2. Groups

The variables independent variables were structured in five groups in such a manner that the tertiary questions could be answered; only those variables relevant for answering the questions were varied (*Table 21*).

7.2.3.3. Test run

Each test run was a mimicking duodenoscope usage while systematically controlling the product and usage variables by means of the groups. The test runs were performed following a strict test run protocol. [Attachment 3]

In between each test run, all stainless-steel construction parts were first cleaned, then disinfected and finally disassembled and sterilized. After sterilization of the construction parts, the dirty side and the clean side basin were immediately covered by the cover to prevent cross-infection of the basins. Before placing the construction parts on the table, a table was disinfected with 70% ethanol. Then the mockup parts were assembled with the aligner and adjustment rings and fixated in the framework with the springs. Depending on the group belonging to the test run, the parts for motion or the parts for pressurization were also assembled. The parts of the construction set-up were assembled using disinfected gloves. The disinfected O-rings were installed in the axle grooves using sterile tweezers.

Prior to the test run, the seals were subjected to the leakage test by pressurizing the dirty side basin to 240 mbar. Then, the test run was started by inserting BHI 500µL BHI broth in the dirty basin. Each test run was 60±2.5 minutes: the maximum time of an ERCP procedure.[59]

7.2.3.4. Sampling- and culture method

After a test run, the clean side of the seal was sampled using fresh BHI broth by indirect immersion of the clean basin. The clean basin cover was lifted and 600µL BHI broth was inserted in the basin. The broth is refluxed (i.e. pipetting fluid in the basin and pipetting it out of the basin) for 10 times. After 200µL BHI broth of the clean side, the contents of the basin were transferred with a pipette to separate TSA plates. Each basin was sampled with a new pipette tip to prevent cross-contamination.

The plates were incubated for 25±0.25 hours at 36±1°C. The CFU on each plate were counted. *K. Pneumonia* on TSA is a flat CFU with a relatively large diameter, with a viscous/mucoid appearance and a yeasty odor. All colonies were checked on their appearances, and those being suspicious for not being *K. Pneumonia* were identified using MALDI-TOF MS (Bruker Nederland BV).

7.2.3.5. Replication & randomization

The test runs were performed up to a sample size of at least 9 seal tightness tests per group. To reduce the influence of construction deviations in each of the 3 mockups of the construction set-up, assembly of the set-up was randomized. Both the combination of the axles-in-block and the locations of the blocks in the framework were randomized in a controlled manner (*Table 23 and Figure 30*).

Table 21. Dependent variables of the controlled experiment

Dependent variable	Level 1	Level 2
Air tightness	Airtight	Air penetration
Bacterial tightness	Bacterial tight	Bacterial penetration

Table 22. Five different groups of the controlled experiment

Group Independent variable	1. Inactive use with lowly-compressed seals	2. Inactive use with highly-compressed seals	3. Pressure on dirty side with highly-compressed seals	4. Rotating axle with highly-compressed seals	5. Rotating, translating axle with highly-compressed seals
Axial displacement of axle (mm)	0	0	0	0	0.375
Effective compression of the installed O-ring	0-13%	13-28%	13-28%	13-28%	13-28%
Pressure at dirty side (mbar)	0	0	16	0	0
Frequency of 90°-dynamic rotational movement (per procedure)	0	0	0	30	30

Table 23. Controlled randomization of the assembly. Top: axle-block combination. Below: block-in-frame combination

Block# Run	Block 1	Block 2	Block 3
1, 4, 7, 10	Axle 1	Axle 2	Axle 3
2, 5, 8, 11	Axle 2	Axle 3	Axle 1
3, 6, 9, 12	Axle 3	Axle 1	Axle 2
Position# Location	**Left**	**Middle**	**Right**
1, 4, 7, 10	Block 1	Block 2	Block 3
2, 5, 8, 11	Block 2	Block 3	Block 1
3, 6, 9, 12	Block 3	Block 1	Block 2

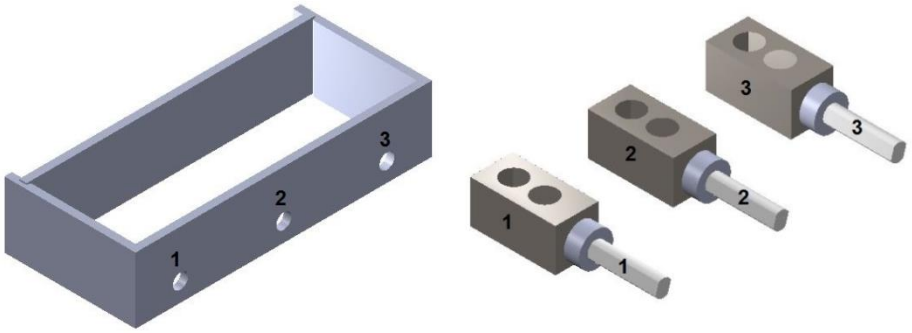

Figure 30. Controlled randomization of assembly of construction set-up; randomization of the axle-block combination and the location of the block in the framework

7.2.3.6. Controls

A negative control was added to exclude an effect of contamination during the test runs on the culture results. For this negative control, a test run was done while having one mockup immersed with fresh BHI broth in both the clean and dirty basin.

Additionally, a second negative control was included to show any effects of contamination of the clean basin through another path than through the O-ring seal. This second negative control was done by performing the experiments on a block without borehole while following the test run protocol (*Figure 29-bottom*). [Attachment 10]

Both negative controls were performed two times for each experimental group on a random axle-in-block combination and test run. The sampling- and culture method for both negative controls was identical as in the mockups linked to a group.

7.2.3.7. Data interpretation

The list below compiles all bacteria interpreted in the MALDITOF-MS results as environmental flora (dermal flora). Since these bacteria have a high likelihood to be cultured as a result of environmental contamination during sampling, the CFU of these bacteria were not counted as penetrated bacteria.

- Micrococcus Leutus
- Staphylococcus epidermis
- Staphylococcus capitis
- Bacillus cereus
- Escherichia coli

7.2.4. Statistical analysis

To be able to answer the tertiary research questions of this Chapter, five null hypotheses were made. Both the air and microbial leak tightness, the results of the systematic investigation, were two-levelled categorical non-parametric data and therefore could be place in contingency tables. With the categorical contingency tables the hypothesis were tested with separate two-way Pearson Chi-Square test of independence while using 95% confidence interval (α-level= 0.05) assuming;

- Non-parametric data;
- Matched data (since all the data was collected from the same experiment set-up);
- The number of degrees of freedom (DOF) is 1 (DOF=(l-1)(s-1)=(2-1)(2-1)=1*1=1, where 'l' is number of levels of the independent parameter and 's' the number of levels of the dependent parameters);
- Sufficiently large sample size

The levels in each group were compared for the presence of a statistically significant difference in the equality of proportions to test the null hypotheses. The upper-tail critical value is 3,841 and the lower-tail critical value is 0,004 of the Chi-Square distribution with 1 DOF using 95% confidence interval ($p<0.05$). [64] The Chi-Square critical values were compared with the upper- and lower-tail critical value.

- The first set of statistical tests was performed to analyze whether air- and bacterial tightness ('air/bacterial penetration' = 1 and 'air/bacterial tight' = 2) and effective compression ('low' = 1 and 'high' = 2) are independent of one another.
    - 1^{st} H0 – Air tightness and the effective compression are independent
    - 2^{nd} H0 – Bacterial tightness and effective compression are independent

    A first statistical test was performed to analyze whether air tightness ('air tight' = 1 and 'air penetration' = 2) and effective compression ('low' = 1 and 'high' = 2) are independent of one another. Chi-Square test of independence was calculated comparing the frequency of airtightness in lowly- and highly-compressed seals.

- The second set of statistical tests were performed to analyze whether bacterial tightness ('bacterial penetration' = 1 and 'bacterial tight' = 2) and usage variables ('variables zero'= 1 and 'variable as in use = 2) are independent of one another.

- 3rd H0 – Bacterial tightness and gauge pressure on the dirty side are independent
- 4th H0 – Bacterial tightness and dynamically rotating loading of the mockups' axles are independent
- 5th H0 – Bacterial tightness and dynamically rotating loading with axial displacement of the mockups' axles are independent

Firstly, a statistical test was performed to analyze whether bacterial tightness ('bacterial penetration' = 1 and 'bacterial tight' = 2) and external pressurization ('variables zero'= 1 and 'gauge pressure' = 2) are independent of one another. Chi-Square test of independence was performed comparing the frequency of bacterial penetration in mockups without and with gauge pressure at the dirty side.

Secondly, a statistical test was performed to analyze whether bacterial tightness ('bacterial penetration' = 1 and 'bacterial tight' = 2) and dynamic rotational loading ('variables zero'= 1 and 'dynamic rotational loading' =2) are independent of one another. Chi-Square test of independence was performed comparing the frequency of bacterial penetration of mockups with a passive axle and with a dynamically rotating axle.

Thirdly, a statistical test was performed to analyze whether bacterial tightness ('bacterial penetration' = 1 and 'bacterial tight' = 2) and dynamic rotational leading with axial displacement ('variables zero'= 1 and 'dynamic rotational loading with axial displacement' = 2) are independent of one another. Chi-Square test of independence was performed comparing the frequency of bacterial penetration of mockups with a passive axle and with a dynamically rotating axle.

- A statistical test was performed to analyze whether bacterial tightness ('bacterial penetration' = 1 and 'bacterial tight' = 2) and effective compression ('low' = 1 and 'high' = 2) are independent of one another. Chi-Square test of independence was performed comparing the frequency of bacterial penetration in lowly- and highly-compressed seals.

7.3. Bacterial tightness of airtight seals

7.3.1. Results of the test runs

The results of the cultures of the test runs of the systematic investigation are reported in tables (*Table 24 and Table 25*) and figures (*Figure 31*, *Figure 32 and Figure 33*). [Attachment 7] All cultures originating from the negative controls showed no growth. [Attachment 7]

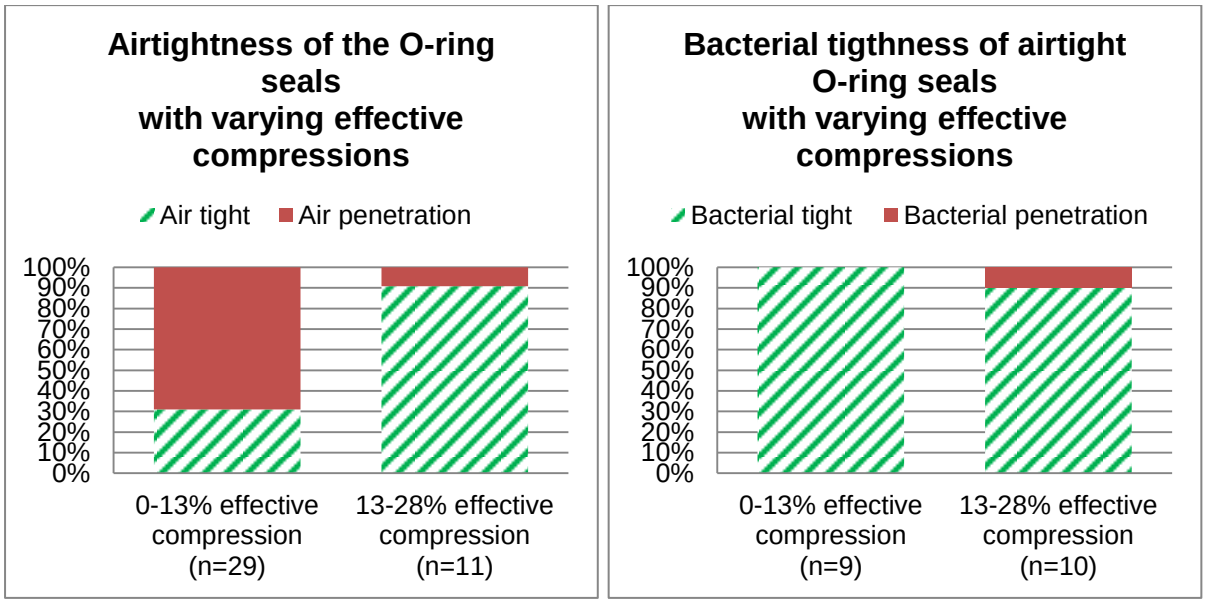

Figure 31. Tightness of the seals with varying compressions. Left: Airtightness. Right: Bacterial tightness.

Table 24. Results of the leakage test (airtightness) with varying seal tightness

	Airtight	Air penetration	Total
0-13% effective compression	9	20	29
13-28% effective compression	10	1	11
Total	19	21	40

Table 25. Presence of microbial leakage. Top: varying seal tightness. Below: varying usage variables.

	Bacterial tight	Bacterial penetration	Total
0-13% effective compression	9	0	9
13-28% effective compression	9	1	10
Total	18	1	19

	Bacterial tight	Bacterial penetration	Total
Inactive use	9	1	10
Pressurization of dirty side	10	1	11
Rotating axle	4	6	10
Rotating, translating axle	3	6	9
Total	26	14	40

7.3.2. Statistical analysis

A significant interaction was found between the seals' airtightness and effective compression ($\chi^2(1, N = 40) = 11.5, p < .05$). [Attachment 8] Highly-compressed seals were more likely to be airtight (91%) than lowly-compressed seals (31%) in passive conditions. On the contrary, no significant interaction was found between the seals' bacterial tightness and effective compression ($\chi^2(1, N = 40) = 0.950, p < .05$). Highly-compressed airtight seals were equally likely to have bacterial penetration (10%) as lowly-compressed airtight seals (0%) in passive conditions.

No significant interaction was found between the seals' bacterial tightness and external compression ($\chi^2(1, N = 21) = 0.00502, , p < .05$). [Attachment 8] Airtight (highly-compressed) seals submitted to gauge pressure at the dirty side were equally likely to have bacterial penetration (9%) seals with no applied gauge pressure (10%). On the contrary, a significant interaction was found between the seals' bacterial tightness and dynamically loading of the axles ($\chi^2(1, N = 20) = 5.50, , p < .05$). [Attachment 8] Airtight (highly-compressed) seals in mockups with dynamical axles were more likely to have bacterial penetration (60%) than in mockups with passive axles (10%).

Also, a significant interaction was found between the seals' bacterial tightness and dynamic rotational loading axle with axial displacement ($\chi^2(1, N = 19) = 6.54, p < .05$). [Attachment 8] Airtight (highly-compressed) seals in mockups with dynamically-translational axles were more likely to have bacterial penetration (67%) than mockups with passive axles (10%). However, airtight (highly-compressed) seals of mockups with dynamically-translating axles were equally likely to have microbial leakage (67%) as seals of mockups with dynamically, non-translating axles (60%), since no significant interaction was found in the seals' bacterial tightness of these two groups ($\chi^2(1, N = 19) = 0.0905, p < .05$). [Attachment 8]

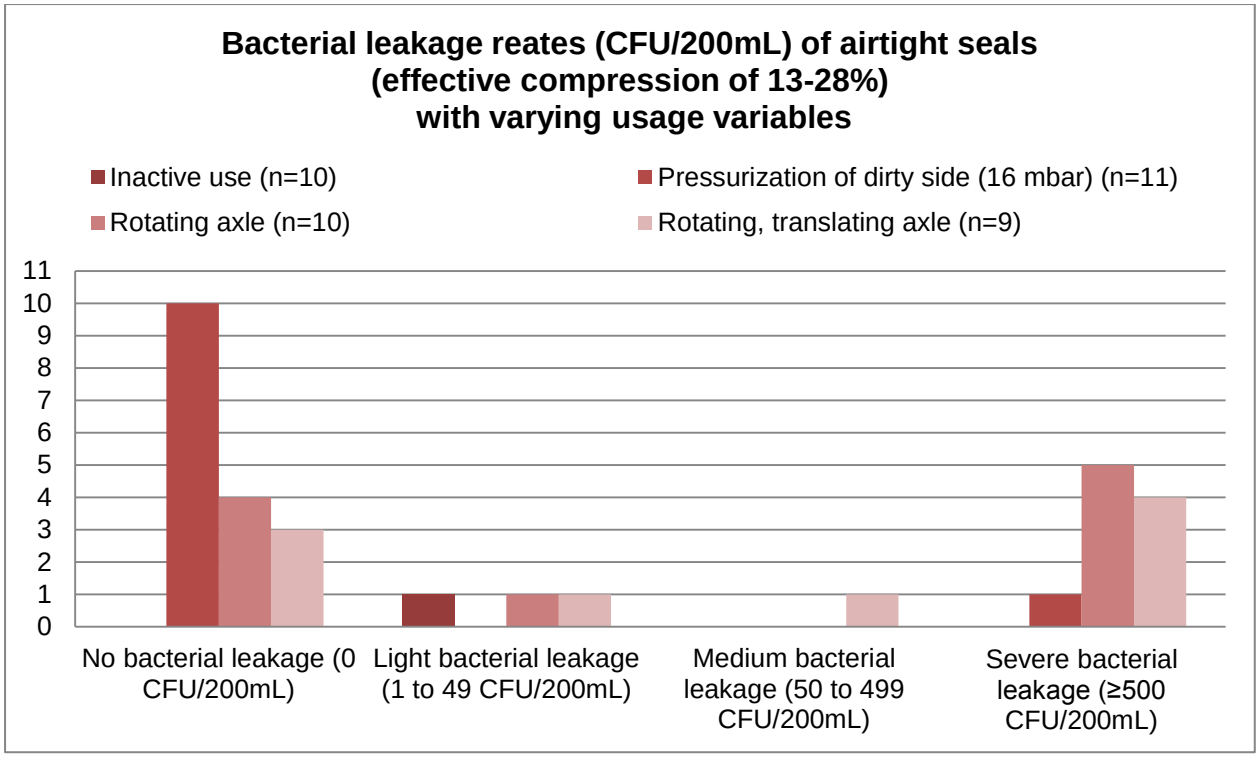

Figure 32. Bacterial leakage rates (CFU/200mL) counted on the culture plates of airtight seals (with an effective compression between 13-28%) with varying usage variables. [Attachment 7]

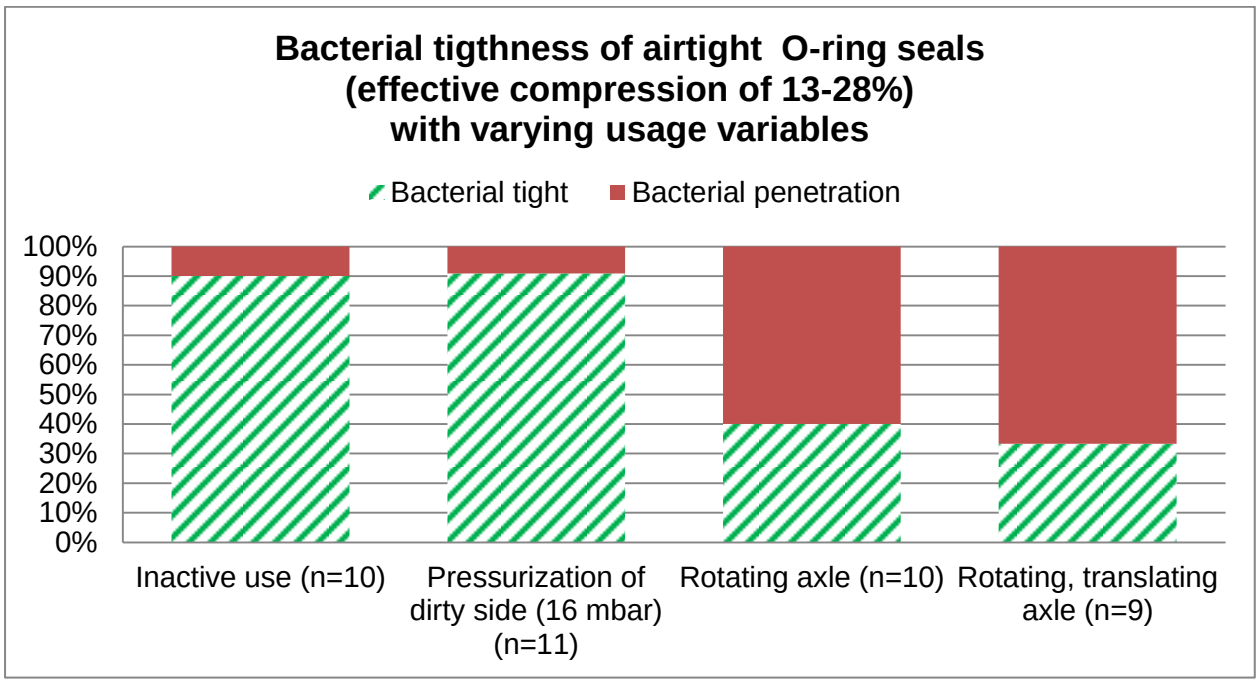

Figure 33. Bacterial tightness of airtight seals (with an effective compression between 13-28%) with varying usage variables. [Attachment 7]

7.4. Discussion and conclusions

*'How should the **product variables** need to be controlled during the systematic investigation such that mockups reproduce the distal O-ring sealing construction in duodenoscopes?'*

The experiment set-up was a triple parallel mockup of the construction in Olympus TJF-Q180V duodenoscopes, to cancel out manufacturing deviations. Most product variables were kept constant in the mockups with the principle to have mockups with more favorable O-ring sealing performance as those in the actual elevator mechanism. Therefore, the mockups were designed and manufactured with the dimensions identical as measured in an Olympus TJF-Q180V, or as recommended as the optimal parameter in literature. The only product variable that was studied in the systematic investigation is the effective compression of the O-ring. Both effective compression ranges –low and high- will also be found in actual instruments since they are an inevitable result of dimensional deviations within the tolerance margins during manufacturing. Varying effective compression is obtained by studying mockups with O-ring with two different cross-sections; a smaller O-ring cross-section resulting in a lower effective compression, and larger O-ring cross-section resulting in a higher effective compression. The two effective compression ranges resulted in 0-13% for the lowly-compressed seal and 13-28% for the highly compressed seal.

*'How should the **usage variables** need to be controlled during the systematic investigation such that a test run mimics usage of the elevator mechanisms of duodenoscopes?'*

The variables during the test runs on the mockups were with inactive usage variables, with gauge pressure on the seals' dirty side, with dynamical axles and with dynamically, translational axles. The gauge pressure on the dirty side was set on the maximum intra-abdominal pressure. In this study, the pressure resulting from reprocessing is not evaluated, since it is expected to be negligible. However, this can be an underestimation; since the elevator recess is flushed with a syringe during manual cleaning, it is possible that pressure is induced on the seal of the water jet from the syringe is exactly targeted on the gap between the lever axle and its bearing hole. To also simulate gauge pressure resulting from reprocessing, the pressure of the cleaning detergent on the O-ring seal during manual cleaning should be studied. The dynamic rotation was estimated to be maximally ¼th of a full rotation (elevator rotation of maximally 90°) and axial displacement was estimated on maximally 0.375mm. Other usage variables, such as the rotary speed, angle of movement, angular displacement and environmental temperature, were kept constant during the test runs. Since the three studies usage variables are set maximum values, the systematic investigation is a worst-case scenario for evaluating the microbial leakage compared to actual ERCP procedures.

Are the mockups a valid reproduction of the elevator construction of Olympus TJF-Q180V duodenoscopes?'

In the evaluation of the bacterial tightness of the O-ring seal in the elevator mechanism of duodenoscopes, the design of the mockups was based on observation of only one Olympus TJF Q180 instruments (Chapter 4.3.1. Dimensions of the distal O-ring sealing construction in duodenoscopes). It can be assumed that these measured dimensions are also valid for other items of these instruments since it is very likely that small tolerances are used by the manufacturer.

Due to variation in the fabrication process, the dimensions of the mockups did not appear to have the exact same dimensions as those of the elevator construction of Olympus TJF-Q180V duodenoscopes. The dimensions of the mockups were similar to those of the elevator mechanism, with exception of the radial clearance (0.11-0.12±0.04 mm in mockups instead of 0.075±0.06mm in actual mechanism), the bearing length (1.33-1.52±0.04 mm in mockups instead of 0.65±0.03mm in actual mechanism) and the stretch (0-3% in mockups instead of 11-17% in actual mechanism). The higher radial clearance in the mockups was expected to have low effect on the sealing efficacy. The longer bearing length and the lower stretch in the mockups were expected to improve the bacterial sealing efficacy. The O-ring seals material type used in the systematic investigation were based on observation of the O-ring in the Olympus TJF-Q180V duodenoscope. However, it was not for sure that the material type of the actual instrument and that of the controlled experiment are identical. To have the similar O-ring material during the controlled experiment as in actual duodenoscopes, manufacturers and repair centers were contacted. Unfortunately, all parties were not able to provide information about the material of the seal. The disadvantage of the design of the experiment set-up

was that the actuation is at the other side of the axle than in actual duodenoscopes. This factor is expected to have no effect on the results.

The mockups are considered as a valid sealing construction to test air- and bacterial penetration, since all variables were closely controlled. Therefore, the mockups look like the elevator construction of Olympus TJF-Q180V, and thus it is likely that bacterial sealing efficacy results found in this experiment are similar to those in duodenoscopes. However, for the reasons above, the mockups are not a full reproduction of the elevator construction.

*'What are the influences of **the product variables** on **the air- and bacterial tightness** of seals (e.g. mockups that are passing the leakage test) with varying usage variables?'*

The airtightness -during the leakage test- was for mockups with lowly-compressed seals 31% and with highly compressed seals 91%. This relationship between the passage of the leakage test and the seal-tightness is dependent was found to be significant. The mockups with inactive usage variables showed bacterial penetration for 0% of the lowly-compressed seals and for 10% of the highly-compressed seals. However, this relationship between microbial leakage and the seal tightness was found to be insignificant.

*'What are the influences of **the usage variables** on **the bacterial tightness** of airtight seals (e.g. mockups that are passing the leakage test)?'*

The controls excluded that cross-infection of the clean side occurred or that sterilization of the setup failed. These negative controls validate that the sampled K. Pneumonia were indeed penetrated through the seal. Bacterial leakage was present in 20% of all the airtight mockups. Bacterial leakage was present in 10% of the airtight mockups with inactive usage conditions, in 9% of the airtight mockups with 16 mbar gauge pressure on the dirty side, in 60%, of the airtight mockups with dynamically axles and in 67% of the airtight mockups with dynamically-translational axles. These results show that microbial leakage in all the sealing groups, but are more likely to occur with dynamic rotational loading. The best-case sealing constructions were used for the test runs with the worst-case usage variables. Therefore, the results of this controlled investigation are an overestimation of the bacterial tightness during maximum use intensity. The results suggest that when the forceps elevator is used during the ERCP procedure, the seals may leak micro-bacteria to the clean side and back to the dirty side. It must be emphasized that the controlled experiment is only an investigation approaching the sealing efficiency against micro-organisms of the distal seal in the Olympus TJF-Q180V in an ERCP procedure. Most ideally, the protocol of this experiment should be applied on the seals in actual duodenoscopes.

If this controlled experiment is continued to obtain a more solid and practically applicable conclusion, the experiment should have a higher number of replications, with at least 5 replications in each cell of the contingency tables. Then it would be interesting to also include a group with varying the angular displacement of the axles; angular displacement is expected to have an influence on the bacterial sealing efficacy since it results in a higher compressed O-ring at one side of the axle and the opposite effect on the other side of the axle. This altered effective compression may allow migration of bacteria to the clean side of the O-ring and thereby increase microbial leakage. Finally, other bacteria than the type used, could give other results, because bacteria could actively migrate across the seal, for example, if having flagella.

Chapter 8. Discussion

8.1. Evaluation of the sealing efficacy against microorganisms of O-ring seals

To have a well-performing O-ring seal, its effective compression must be within a particular range. The sealing surface determines the sealing performance; therefore, the effective compression (e.g. seal tightness) must be in a particular range to provide sealing of air and fluid. Optimal effective compression ranges -as commonly advised in literature- are for dynamic O-ring seals 6-20% and for static O-ring seals 15-30%. Even though effective compression defines the seal performance, other factors must also be beneficial to have a performing seal; the stretch must be low (maximally 4%), lead-in chamfers must be present for installation, a smooth seal-contact material surface and the seal must have periodical the maintenance.

O-ring sealing performance can be assessed by tightness for gasses and fluids. Even for tight seals, leakage is present in small amounts: due to the surface roughness of the housing and O-ring (roughness of a new O-ring visualized on the cover page), non-contact areas can exist only visible at a high magnification.. [5] In medical instruments O-ring seals may be used in direct contact with patient material containing micro-organisms. Theoretically, micro-organisms will not leak through seals if there is no leakage of fluids and gasses since micro-organisms' sizes are larger than singular molecules; for the leakage of bacterial marginal gaps are needed of at least 0.5µm-1.0µm. [56] However, it is important to keep in mind that bacteria are living organisms; they might actively migrate and promote leakage, and moreover, one leaked bacteria could duplicate, while one molecule of air of fluid just stays one molecule. It could be a crucial misconception that air- and fluid leakage and microbial leakage are inherent; absence of visible air leakage does not necessarily have to exclude leakage of microorganisms. Microbe leaking O-rings may especially be a problem for medical instruments that are reprocessed with chemical disinfection, since with this method is a reduction of vivid microbes by contact between microbes on the instruments' surfaces and disinfectants, unlike of thermal disinfection and sterilization.

The clearances in duodenoscopes at the dirty side of the distal O-ring seal are small voids that might harbor microorganisms. Moreover, if the O-ring leaks microorganisms to the clean side of the construction during its life cycle, the clean side of the axle and lever recess could act as a micro-reservoir for bacteria. The microorganisms could survive in small voids and since migration of microorganisms back to the elevator recess cannot be excluded if contamination of the clean side can occur, concealed microorganisms in the contaminated clean side of an O-ring construction can consequently be discharged into the patient during an ERCP procedure. Entrapped bacteria have a low chance of to be killed during the reprocessing procedure since the O-ring seals these contaminated surfaces against fluids.

Theoretically, the O-ring sealing mechanism in the distal tip of duodenoscopes is air and fluid tight to seal the elevator wire channel. The sealing efficiency of the O-ring seal should be tested during leakage test conducted with manual and automatic cleaning by pressurizing the interior of the instrument. Since the leakage test defines the evaluation of the sealing performance between successive uses, the space adjacent to the seal must be pressurized to a sufficiently high pressure during the leakage test. Results of this study indicate that there is no air flow at the end of the elevator wire channel during the leakage test, what would lead no pressurization of the lever recess at all. The effectiveness of the leakage test on the O-ring is highly questioned. An evaluation of one Olympus TJF-Q180V duodenoscope indicated that the stretch is between 11 and 17%; higher than the literature-advised range for seals larger than 1.0mm of 1-4%. Highly stretched O-rings are more prone to show at an early stage material failure. However, a covering conclusion cannot be made, since the duodenoscope's distal O-ring is with its 0.5mm smaller than 1.0mm. The effective compression measured of 0 to 25% seems to be in the advised range.

Microbial leakage through the O-ring seal in the distal tip of duodenoscopes to the clean side of the construction can only be detected if the distal tip of the instrument is disassembled. For this reason, microbiological screening of the clean side of the construction was started in an independent repair center. However, only three duodenoscopes were repaired over the study period: a much lower number than expected prior to the start of the study. This can be explained by the recall Olympus of

their TJF-Q180V models; since clinics sent their duodenoscopes for repair to Olympus instead of an independent repair center. No contamination was found on the clean side of the distal seal in duodenoscopes during repair.

Mockups of the distal O-ring seal in the elevator mechanism of Olympus TJF-Q180V duodenoscopes were submitted to leakage tests and test runs mimicking usage in order to systematically study bacterial leakage or airtight seals. The results showed that bacterial penetration was present in airtight mockups of all groups and was significantly more when the axles were dynamically rotated. These results suggest that in actual duodenoscopes the O-ring seal in the elevator mechanism of Olympus TJF-Q180V duodenoscopes is an efficient shield for micro-organisms in passive variables, but when the forceps elevator is used during ERCP procedures, the seals may leak micro-bacteria to the clean side. The best-case sealing constructions were used for the investigation, with the worst-case usage variables; therefore, the results are an overestimation of the bacterial tightness during maximum use intensity. This means that if the distal O-ring seal in actual duodenoscopes is optimized to a best-case seal by controlling close tolerances, the seal may leak microorganisms during intensive ERCP-procedures. It should be emphasized that the systematic investigation is approaching the sealing efficiency against micro-organisms of the distal seal in the Olympus TJF-Q180V in an ERCP procedure, and therefore is not a fully valid replication of this effect in actual duodenoscopes.

An ideal design of the duodenoscopes' distal sealing would be to have a seal of which all the surfaces of the O-ring seal and its housing can be disinfected; both at the dirty and clean side. Unfortunately, this is not feasible for reprocessing departments; disassembly of the elevator axle from its housing will be too time-consuming. A good step forwards would be to evaluated the seal performance thoroughly between each cycle of use by ensuring leakage test effectiveness of the distal O-ring seal, for example by facilitating lever recess pressurization by creating a direct inlet as suggested in 'Chapter 5. Computational analysis of the (air) leakage test during manual cleaning'. Moreover, the O-ring sealing performance can be kept optimal, by revising and replacing the seal routine basis.

8.2. Limitations of the studies

The main limitation of the studies in this thesis was the low number of replications on several aspects of the evaluation. This was due to restrictions in the availability of duodenoscopes that could be disassembled to evaluate the sealing mechanisms. Firstly, the number of instruments included in the surveillance study was much lower than expected. Due to this limitation, no conclusion could be given about the occurrence of leakage of bacteria in the seals of actual new model duodenoscopes. Besides, the number of replications of measurements of the systematic investigation should be higher to increase statistical power; for the statistical test applied, a minimum of 5 replications for each cell of the contingency table would have been preferable. This is not applicable each cell of the systematic study. By increasing the number of replications, the conclusion will have a higher statistical power.

This systematic investigation is a case study of the bacterial sealing efficacy of the O-ring seals in Olympus TJF-Q180V. Even though the mockups used are highly similar to the distal duodenoscope's sealing construction, it is not a full replication, and thereby not a fully valid representation.

8.3. Recommendations for succeeding studies

To be able to give a more decisive conclusion about the bacterial tightness of the O-ring seals in the elevator mechanism of duodenoscopes, studies are needed with a higher the external validity of the studies in this thesis; clinical data needs to be added to evaluate the microbial sealing efficacy of the seal. For the surveillance of microbial leakage through the seal during disassembly of duodenoscopes, the protocol of the study of Chapter 6 can be applied. [Attachment 9] Ideally, data collected from the sampling of instrument surfaces is directly done after ERCP procedure and after reprocessing. Unfortunately, since disassembly of the distal tip is needed, this sampling cannot take place in clinics. Largely-scaled surveillance studies could be started at repair centers of third party repair centers and manufacturers during the revision of the endoscope.

Also, the effectiveness of the leakage test on the distal O-ring in duodenoscopes could be studied further. If the pressure in the elevator lever recess in distally-sealed models duodenoscopes is studied with pressure sensors, the effectiveness of the leakage test can be profoundly evaluated.

Besides, the controlled experiment evaluating bacterial leakage through O-rings can be extended by replicating the experiment and adding more groups with other factors that can possibly influence microbial leakage. This would add valuable information to give researchers and developers of medical instruments more insight to the microbial sealing behavior of O-rings. Standard O-ring sizes listed in ISO3601-2 can be used as case studies. [52] A stepper motor can be integrated into the experimental set-up to standardize the motion pattern of the test runs. [Attachment 10]

Finally, an inventory of currently used O-ring seals in medical devices in direct contact with patient material, other than in distally-sealed duodenoscopes, could be made. Other instruments with O-rings in direct contact with patient material should be traced, in order to study the microbial sealing performance and any related risk of infection for patients.

Chapter 9. Conclusions

All of the chapters in this thesis had the aim to give partially answer the research question *'What is the bacterial tightness of the O-ring seal in the distal tip of pre-recall Olympus TJF-Q180V duodenoscopes?*'.

The results of the systemic study indicated that static O-ring seals integrated into dental implants showed bacterial penetration (airtightness was not tested) and that reprocessed, static O-ring seals in dialyzers were associated with hospital infections. An investigation of a duodenoscope showed brownish debris on the clean side of the distal O-ring. These findings may suggest that O-rings may leak bacteria and their constructions may be difficult to effectively reach during reprocessing. Nevertheless, in this thesis, microbial leakage in duodenoscopes in clinical practice has not been found in the surveillance study.

The sealing efficacy was evaluated in the distal O-ring seal in the elevator lever axle of duodenoscopes. After measuring one Olympus TJF-Q180V, the factors of the sealing construction that might affect the sealing interface were evaluated, indicating that all factors seem in coherence with the recommended values with exception of the too high stretch of 11-17%. Besides, it was found that the leakage test possibly does not pressurize the space adjacent to the O-ring, what would have as consequence that the sealing performance is not tested. Bacterial leakage was evaluated in a systematic investigation; mockups of the elevator sealing construction were subjected to a controlled investigation while simulating instrument use of an ERCP procedure. Significant microbial leakage was found in mockups with the dynamic rotational loading of the axles. The results suggest the distal O-ring seals in duodenoscopes might potentially leak microorganisms if the forceps elevator is extensively used. However, since the mockups used in the controlled investigation are not a full replication of the elevator mechanism of the instrument, these results cannot be used as proving that this microbial leakage is actually present in duodenoscopes.

It was found that microbial leakage can occur even though the seal passed a leakage test of 240 mbar. The results indicate that an airtight seal does not necessarily is a bacterial tight seal. Medical designers, manufacturers and safety controllers should take knowledge of this insight that airtight O-ring seals - designed in accordance with the standards- might leak microorganisms. Reuse of instruments with O-rings in direct contact with patient material might thereby pose a risk to infection safety for patients since the microorganisms trapped in the O-ring sealing will be hard to effectively kill during reprocessing.

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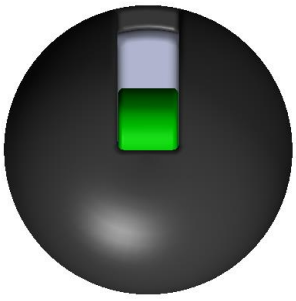
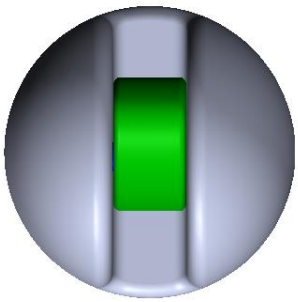
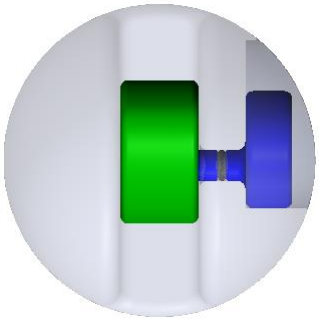
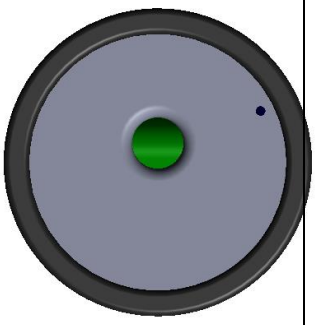
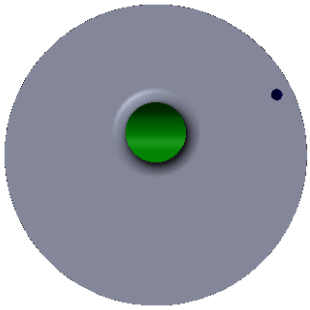
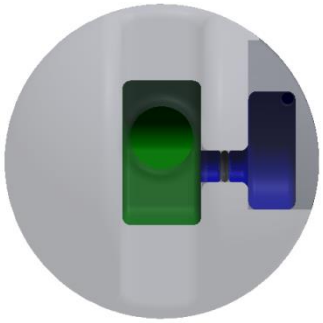
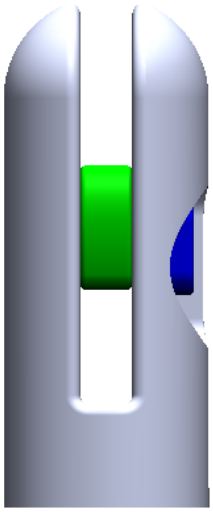
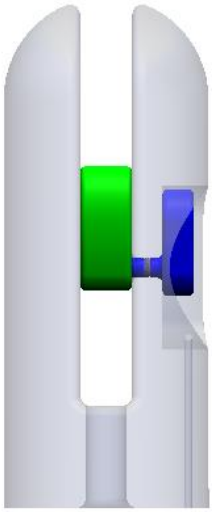
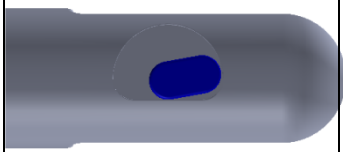
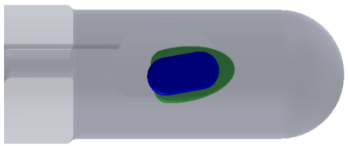
Attachments

Attachement 1 - Thee plane projections of the distal tip of distally sealed duodenoscopes

Three plane projections of the distal tip

	With the distal cap	Without the distal cap	With transparent tip body
Proximal view			
Distal view			

Top view			
Lateral left view			

Attachment 2 - Dutch manual for 'Screening of microbial contamination leaked through the distal seals in duodenoscopes'

De plekken in de ERCP-endoscopen waar de kweken afgenomen gaan worden verschillen voor de modellen met een **open tangenliftkanaal** en de **gesloten tangenliftkanaal**. Hieronder kunt u voor beide modellen de locaties vinden waarvan een kweek genomen moet worden, en welke ERCP-scopen onder de open en welke onder de gesloten modellen vallen.

Open modellen

Olympus TJF 145

Olympus TJF 160(V)F

1. Onderop de tangenlift als de lift naar boven staat

Direct na het verwijderen van de (afhaalneembare) distale kap:

2. In de kap
3. Zijkant van de tip op het staal
4. Onderop de tangenlift tussen de groef van de as

Gesloten modellen

Olympus TJF Q180V

Pentax ED-3490TK

Pentax ED-3670TK

1. Onderop de tangenlift als de lift naar boven staat

Indien (vaste) distale kap verwijderd wordt, direct na het verwijderen van de kap:

2. Zijkant van de tip op het staal
3. Onderop de tangenlift waar de kap voor zat en tussen de groef van de as

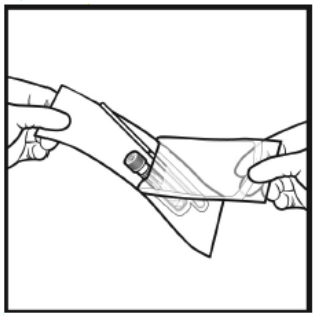
Indien (vaste) metalen afdekkapje verwijderd wordt, direct na verwijderen van het kapje:

4. Op de binnenkant van het stalen kapje
5. Aandrijfruimte van de hefboom van de tangenlift

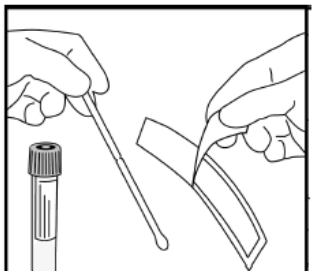
Als u een vraag heeft, bel (0658593279) of mail (i.n.brouwer@umcg.nl) gerust
Contactpersoon: Ilona Brouwer

Stappen

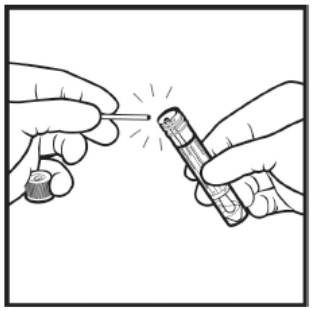
1. Pak een nieuwe envelop met daarin een kweek-kit
2. Haal de kweek-kit met het stickervel uit de envelop en de plastic zak
3. Neem het het tooltje waarmee de distale kap verwijdert wordt en de distale kap af met een doekje met alcohol erop
4. Pak een nieuw kweekbuisje uit en kweekkit en haal hem uit de verpakking

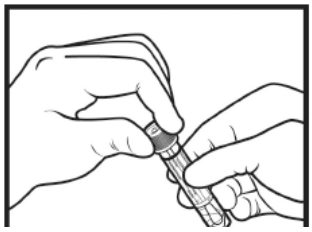


5. Pak het wattenstaafje en veeg hem over het oppervlakte van de onderkant van de tangelift; doe dit zonder andere oppervlaktes aan te raken

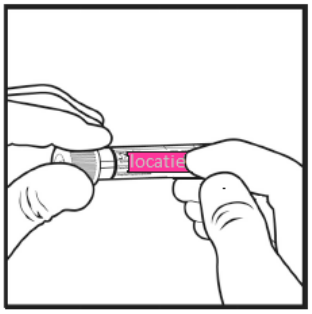


6. Maak het kokertje open, stop het wattenstaafje in de vloeistof, breek het einde van het wattenstaafje af, en sluit de kokertje goed af; **niet het staafje vast houden onder de rode rand**





7. Plak de sticker met de afgenomen locatie op het kokertje

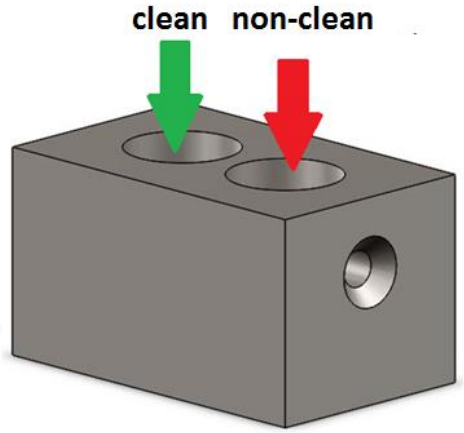


8. Herhaal stap 4 t/m met 7 voor de oppervlaktes uit de rest van het lijstje op de andere kant van deze handleiding
9. Stop de kokers in de kweek-kit; **vergeet niet de kokers goed dicht te doen en stickers te plakken**
10. Vul het aanvraagformulier in, alleen informatie op de gele lijnen hoef ingevuld te worden
11. Sluit de kweek-kit, stop de kweek-kit in de plastic zak, plak de plastic zak dicht door de strip van de zelfklevende rand af te halen, stop ook het aanvraagformulier in de enveloppe en doe het ristje van de envelop helemaal dicht
12. Doe de envelop op de post, bij voorkeur dezelfde dag en uiterlijk de volgende dag; een postzegel is niet nodig

Attachment 3 - Experiment protocol

Step 1: preparation

First, the mock-ups are prepared as required for the experiment run. The thin and thick axles are assembled with the O-ring and closing ring around the thick axle, such that the distal end of the axle is aligned with the aligner in the clean basin.(Protocol-Figure 1) If no axial displacement is required during the experiment, setting rings without angled surface are used. If axial displacement is required during the experiment, setting rings with angled surface are used. Then, a Viton 9.0x1.5mm O-ring is installed in the groove of the pump adapter.



Protocol-Figure 1. The basin block with a clean basin and a dirty basin.

Step 2: reprocessing

The 3 sets of mock-ups are washed with distilled water by insertion of 800µL purified water in each of the basins with a new pipette tip and then the content of each basin is refluxed for ten times: first the clean sides are refluxed and then the dirty sides. The cleaning water is removed with a new pipette tip, and the basins are washed again with the same cleaning method. Then, the mock-ups are disinfected by insertion of with 800µL 70% ethanol in each of the basins with a new pipette tip, and then refluxed it for ten times: first the clean sides are refluxed and then the dirty sides. The 70% ethanol is removed with a new pipette tip. After disinfection, the parts are put in a stainless steel fine meshed tray without crossing over surfaces, and the tray is sterilized in the autoclave at 121±1°C for 15 minutes.

Step 3: assembly

After sterilization of the construction parts, a table surface is disinfected with 70% ethanol, the basin blocks are positioned on the table and the basins of the blocks are immediately covered by the covers. Then, a cup with 3 sterilized O-rings with the required size for the experiment run is opened. Each O-ring is installed in the groove of the small axle with a sterile tweezers. After installation of an O-ring in the groove of the small axle, the axle is directly inserted in the borehole of the basin blocks (*Protocol-Figure 3*); the installation of the axles is randomized (*Protocol-Table 1*). If axial displacement is required during the experiment, setting rings with an angled surface are assembled with the longest side aligned parallel with the axis the ball bearing. (*Protocol-Figure 2*) After sliding the springs around the axles, the axles are inserted in the hole of the support construction. (*Protocol-Figure 4*) The side panel is slide in the support construction and is aligned parallel with the back basin blocks, such that the spring is loaded. (*Protocol-Figure 5*, *Protocol-Figure 4*) If pressure is required during the experiment, the fluid column is placed in the dirty basin and sealed with Orin wax. (*Protocol-Figure 5*) If rotation of the axles is required, the axles are manually turned using the following pattern: 1 second movement of 90° forth, is followed after 1 minute break by a 90° backwards 1 second movement. If axial displacement is required, the ball bearings are inserted. (*Protocol-Figure 2*)

Protocol-Table 1. Randomized assembly for each run of the experiments

Run Block#	Block 1	Block 2	Block 3
1, 4, 7, 10	Axle 1	Axle 2	Axle 3
2, 5, 8, 11	Axle 2	Axle 3	Axle 1
3, 6, 9, 12	Axle 3	Axle 1	Axle 2

Run Location	Left	Middle	Right
1, 4, 7, 10	Block 1	Block 2	Block 3
2, 5, 8, 11	Block 2	Block 3	Block 1
3, 6, 9, 12	Block 3	Block 1	Block 2

Step 4: immersion

After assembly of the construction, the cover of the dirty basin is lifted and 500 µL bacteria suspension *Klebsiella pneumonia* is inserted. (Protocol-Figure 1) If external pressure is required, the fluid columns are slowly filled with *Klebsiella pneumonia* suspension until the 5th scale. The experiment time is started at the moment of the insertion of the bacteria suspension.

Step 5: experiment run

The construction is kept immersed for 1 hour at room temperature with variable variables, depending on the experimental conditions. (*Protocol-Table 2*)

Protocol-Table 2. Maximum values

Group Value variable	Passive with loose seal (P-loose)	Passive with tight seal (P-tight)	Active pressure with tight seal (A1-tight)	Active rotation with tight seal (A2-tight)	Active rotation & axial displacement with tight seal (A3-tight)
O-ring size (mm)	2,31x0,46	2,31x0,56	2,31x0,56	2,31x0,56	2,31x0,56
Axial displacement of axle (mm)	0	0	0	0	0.375
Pressure at dirty side (mbar)	0	0	16	0	0
Frequency of 90°-dynamic rotational movement (per procedure)	0	0	0	30	30

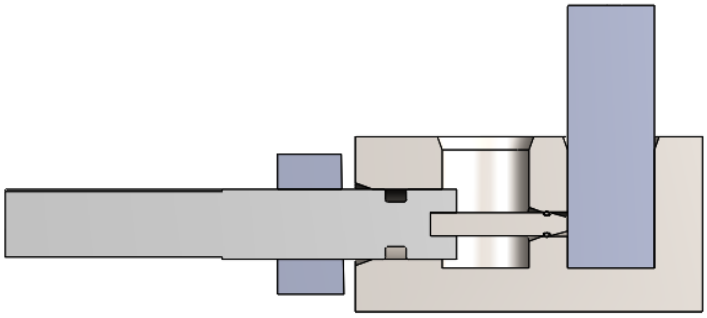
Step 6: bacterial tightness evaluation

After the experiment run with the required conditions, the clean basin is lifted and rinsed with 600 µL of fresh broth by refluxing the broth in a basin for ten times. (Protocol-Figure 1) From the clean basins 200 µL was transferred with a pipette to separate TSA plates. Each clean basin is sampled with a separate, new pipette tip. The plates are incubated for 25±0.25 hours at 36±1°C. The colonies are identified using Maldi-Tof MS.

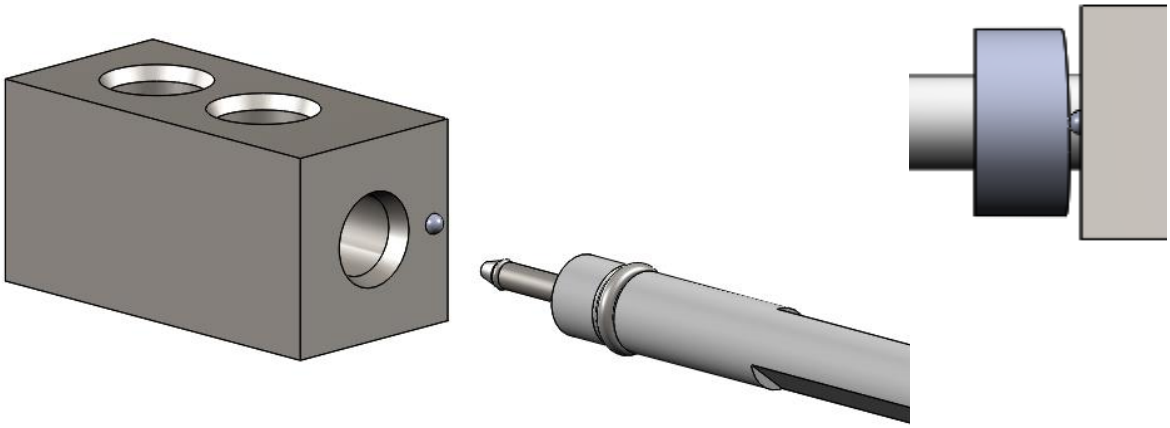
Step 7: leakage testing

After leakage evaluation the mock-up constructions are submitted to a leakage test. The 13 mm-tube of the adjusted manual sphygmomanometer is attached to the pump adapter on top of the dirty basin. The dirty basin is pressurized to 300 mmHg by inflation with the hand cuff. All the time the pressure is inspected on the clock. If the pressure starts dropping down, the pressure of leakage is registered.

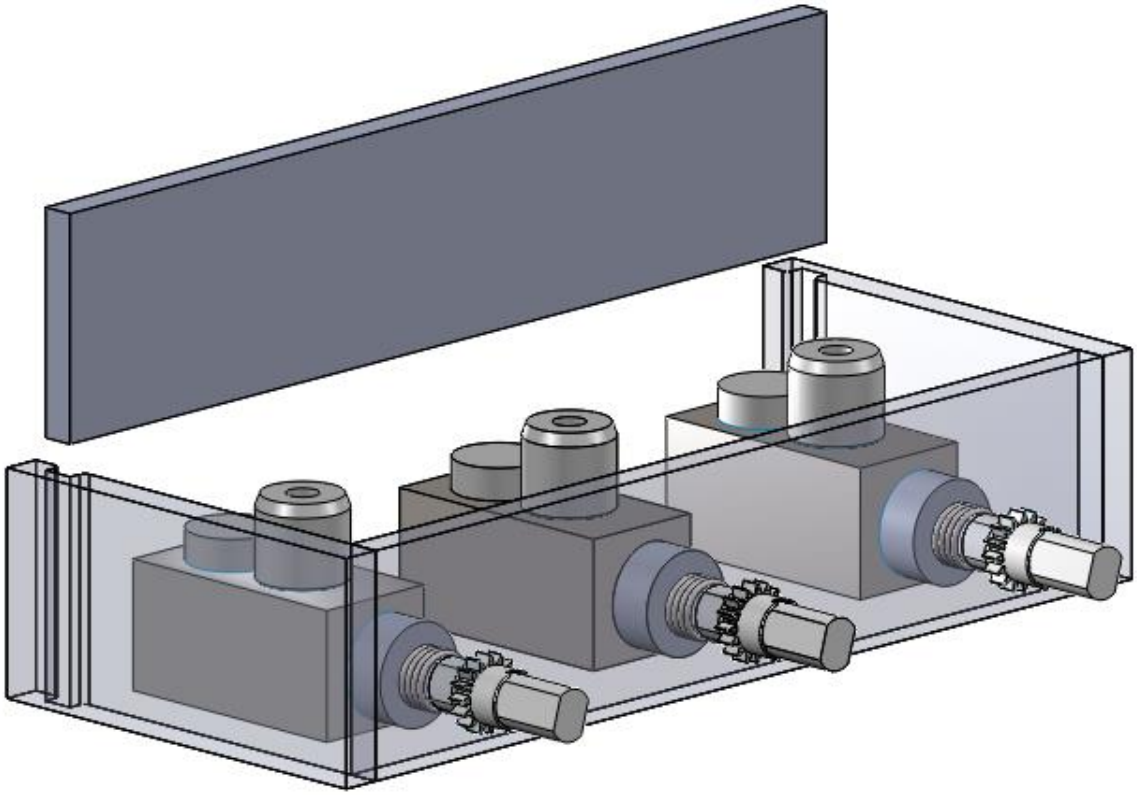
Step 1 up to 7 are repeated with experiment condition of the five groups (P-loose, P-tight, A1-tight, A2-tight and A3-tight) up to a replication of at least nine leakage-test passing mock-ups for each group. (*Protocol-Table 2*) As negative controls, two controls are randomly performed for each group: once with broth both in the clean and dirty basins, and once with a construction with no borehole.



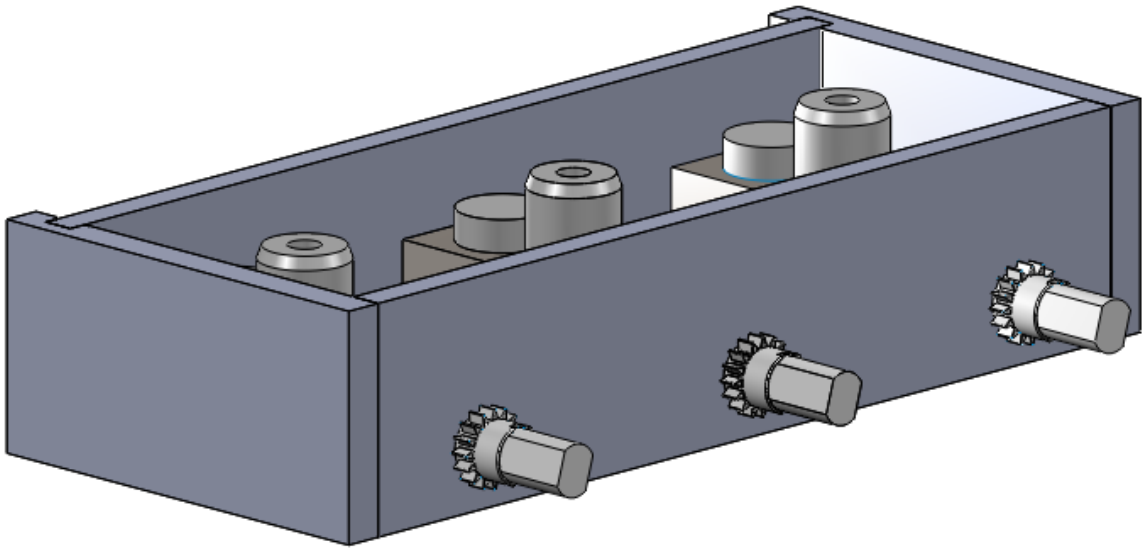
Protocol-Figure 2. The axles are inserted in the boreholes of the basin block such that the distal end of the axle is aligned with the aligner in the clean basin



Protocol-Figure 3. If axial displacement is required during the experiment, the setting ring with an angled surface is assembled with the longest side parallel to the axis of the ball bearing.



Protocol-Figure 4. The side panel is slide in the support construction is parallel aligned with the back basin block such that the spring is loaded.



Protocol-Figure 5. If pressure is required during the experiment, the pump is connected to the pump connectors with tubes. If rotation is required during the experiment, the gears are connected to the axles.

Attachment 4 - Email contact with a physician about ERCP procedures

Beste Ilona,

In antwoord op je vragen. Hopelijk helpen de antwoorden je iets verder.

1) De tijd dat een ERCP scoop in de patient is, is bij ons gemiddeld 60 minuten. Dat is misschien meer dan je in de literatuur hebt gevonden maar is te verklaren doordat we een relatief moeilijke (academische) patientenpopulatie hebben met dientengevolge lastige procedures.

2) Geen idee, meten we niet, heb ik geen info over.

3) Kan enorm varieren, soms 1 wissel, bij sommige ERCPs wel 10 wissels. Gemiddeld misschien 3-4 keer, maar 4 is zeker niet het maximum dat voorkomt.

4) Kan ook enorm varieren, is strict genomen niet gebonden aan een maximum, je schatting lijkt me redelijk.

Groetjes, Jan Jacob

Van: Brouwer, IN (mmb) Verzonden: vrijdag 13 mei 2016 14:15 Aan: Koornstra, JJ CC: Lokate, M (mmb)

Onderwerp: onderzoek tangenlift

Beste Jan Jacob,

In het kader van mijn afstudeeronderzoek bij de afdeling Medisch Microbiologie – Infectie Preventie zou ik graag praktijk terugkoppeling van een MDL-arts krijgen over een aspect uit mijn onderzoek. Van mijn begeleidster Mariëtte Lokate heb ik u doorgekregen als geschikt contactpersoon.

Ik studeer Biomedische Techniek aan de TU in Delft, en ben een experiment aan het doen naar de patiëntveiligheid (microbiologisch) van de constructie van de tangenlift van nieuwe modellen ERCP-scopen, met behulp van een nagebootste constructie waarin allerlei variabelen naar wens ingesteld kunnen worden. Graag zou ik feedback hebben over de gekozen experiment omstandigheden. Deze moeten zo goed mogelijk overeenkomen met de omstandigheden bij ERCP ingegrepen. Ik heb de waardes 1 t/m 3 uit de literatuur gehaald en waarde 4 ingeschat, feedback uit de praktijk als toevoeging zou heel fijn zijn. Zou u deze waardes kunnen langsgaan en beoordelen of ze overeenkomen met uw ervaring? Het gaat om maximum waardes en er hoeft geen onderscheid gemaakt worden tussen verschillende type ERCP ingrepen.

1. Totale tijd dat de scoop ingebracht is tijdens een ERCP ingreep – vaak niet langer dan een half uur

2. Intra-abdominale druk aanwezig? – Ja, 16 mbar

3. Aantal accessoires-wissels bij een ERCP ingreep – maximaal 4 keer

4. Aantal aanpassingen (naar boven of naar beneden doen) van de tangenlift per ERCP ingreep – maximaal 17 keer per ERCP ingreep (4 aanpassingen bij positioneren guide wire, 12 aanpassingen guide wire bij de 3 wisselingen van de accessoires, en 1 aanpassing bij het verwijderen van de guide wire).

Hopelijk kunt u tussen de werkzaamheden door een moment vinden naar deze mail te kijken. Als u een vraag of onduidelijkheid heeft, laat het gerust weten!

Vriendelijke groet, Ilona Brouwer

Attachment 5 - Measured dimensions of the mock-ups

Overview of the dimensions of the axles of the mock-up construction and the deviation of the maximum tolerance

Dimension of the axles	Symbol	Measurement method	Intended dimensions set-up (mm)	Measured dimensions set-up axle 1 (mm)	In tolerance?
Piston diameter	d9	Caliper (±0.03mm)	3,05±0.01	2,97	No; -0,08mm
Housing inside diameter for piston application	d3	Caliper (±0.03mm)	2,40±0.01	2,38	No; -0,02mm
Width of the O-ring housing	dx	Caliper (±0.03mm)	0,80±0.01	0,81	Yes
Bearing length 2	M2	Caliper (±0.03mm)	2,00±0.01	2,00	Yes

Dimension axle2	Symbol	Measurement method	Nominal size (mm)	Real size (mm)	In tolerance?
Piston diameter	d9	Caliper (±0.03mm)	3,05±0.01	2,97	No; -0,08mm
Housing inside diameter for piston application	d3	Caliper (±0.03mm)	2,40±0.01	2,37	No; -0,03mm
Width of the O-ring housing	dx	Caliper (±0.03mm)	0,80±0.01	0,81	Yes
Bearing length 2	M2	Caliper (±0.03mm)	2,00±0.01	2,00	Yes

Dimension axle3	Symbol	Measurement method	Nominal size (mm)	Real size (mm)	In tolerance?
Piston diameter	d9	Caliper (±0.03mm)	3,05±0.01	2,97	No; -0,08mm
Housing inside diameter for piston application	d3	Caliper (±0.03mm)	2,40±0.01	2,31	No; -0,09mm
Width of the O-ring housing	dx	Caliper (±0.03mm)	0,80±0.01	0,80	Yes
Bearing length 2	M2	Caliper (±0.03mm)	2,00±0.01	2,00	Yes

Overview of the dimensions of the blocks of the mock-up construction and the deviation of the maximum tolerance

Dimension block1	Symbol	Measurement method	Nominal size (mm)	Real size (mm)	In tolerance?
Bore diameter for piston application	B	Optical measurement (±0.01mm)	3,2±0.01	3,195	Yes
Bearing length 1	M1	Caliper (±0.03mm)	4,95±0.01	4,96	Yes
Calculated bearing length	M	Calculated (=M1-M2-dx-1.50)	0.65±0.03	0.64	Yes

Dimension block2	Symbol	Measurement method	Nominal size (mm)	Real size (mm)	In tolerance?
Bore diameter for piston application	B	Optical measurement (±0.01mm)	3,2±0.01	3,205	Yes
Bearing length 1	M1	Caliper (±0.03mm)	4,95±0.01	4,95	Yes
Calculated bearing length	M	Calculated (=M1-M2-dx-1.50)	0.65±0.03	0.64	Yes

Dimension block3	Symbol	Measurement method	Nominal size (mm)	Real size (mm)	In tolerance?
Bore diameter for piston application	B	Optical measurement (±0.01mm)	3,2±0.01	3,200	Yes
Bearing length 1	M1	Caliper (±0.03mm)	4,95±0.01	4,95	Yes
Calculated bearing length	M	Calculated (=M1-M2-dx-1.50)	0.65±0.03	0.65	Yes

Attachment 6 – Stretch and effective compression calculations

Measured in Olympus TJF-Q180V

Dimension	Measured value	Symbol	Calculation	Min. and max. value including measurement accuracies
Inner diameter		d1	2,1	2,07
				2,13
Cross section		d2	0,5	0,47
				0,53
Piston diameter	3,05	$d9_{min}$	-	3,02
		$d9_{max}$	-	3,08
Bore diameter for piston application	3,2	$d4_{min}$	-	3,17
		$d4_{max}$	-	3,23
Housing inside diameter for piston application	2,4	$d3_{min}$	-	2,37
		$d3_{max}$	-	2,43
Radial housing depth	0,4	t_{min}	(d4min-d3max)/2	0,37
		t_{max}	(d4max-d3min)/2	0,43
Diametrical stretch inside diameter O-ring installed (mm)	0,3	s_{min}	d3min-d1max	0,24
		s_{max}	d3max-d1min	0,36
Percentage of inside diameter stretch (%)	14,30%	S_{min}	$(s_{min}/d1_{max})*100[\%]$	11,27
		S_{max}	$(s_{max}/d1_{min})*100[\%]$	16,90
Percentage of O-ring cross-sectional reduction resulting from diametric stretch (%)	9,94	R_{min}	$0,56 + 0,59(\min S) - 0,0046(Smin)^2$	6,62
		R_{max}	$0,56 + 0,59(Smax) - 0,0046(Smax)^2$	9,22
Cross section installed O-ring (mm)	0,45	$d2^*_{min}$	d2min –d2min(Rmax/100)	0,43
		$d2^*_{max}$	d2max –d2max(Rmin/100)	0,49
Percentage of effective O-ring cross-section compression (%)	11,10%	C_{min}	$(d2^*_{min}-t_{max})/d2_{min}^*$	-0,78
		C_{max}	(D2*max-tmin)/D2*max	25,24

Design for mockups with a lowly-compressed seal

Dimension	Symbol	Calculation	Min. and max. value including measurement accuracies
Inner diameter	d1	2,31	2,30
			2,32
Cross section	d2	0,46	0,45
			0,47
Piston diameter	d9min	3,05	3,04
	d9max	-	3,06
Bore diameter for piston application	d4min	3,2	3,19
	d4max	-	3,21
Housing inside diameter for piston application	d3min	2,4	2,39
	d3max	-	2,41
Radial housing depth	tmin	(d4min-d3max)/2	0,39
	tmax	(d4max-d3min)/2	0,41
Diametrical stretch inside diameter O-ring installed (mm)	smin	d3min-d1max	0,07
	smax	d3max-d1min	0,11
Percentage of inside diameter stretch (%)	Smin	(smin/d1max)*100[%]	3,02
	Smax	(smax/d1min)*100[%]	4,74
Percentage of O-ring cross-sectional reduction resulting from diametric stretch (%)	Rmin	0,01 + 1,06(Smin) − 0,1 (Smin)2	2,30
	Rmax	0,01 + 1,06(Smax) − 0,1 (Smax)2	2,79
Cross section installed O-ring (mm)	d2*min	d2min –d2min(Rmax/100)	0,44
	d2*max	d2max –d2max(Rmin/100)	0,46
Percentage of effective O-ring cross-section compression (%)	Cmin	(d2*min-tmax)/d2min*	6,28
	Cmax	(D2*max-tmin)/D2*max	15,07

Actual mockups with lowly-compressed seal

Dimension	Symbol	Calculation	Min. and max. value including measurement accuracies
Inner diameter	d1	2,31	2,30
			2,32
Cross section	d2	0,46	0,45
			0,47
Piston diameter	d9min	-	2,96
	d9max	-	2,99
Bore diameter for piston application	d4min	-	3,19
	d4max	-	3,21
Housing inside diameter for piston application	d3min	-	2,28
	d3max	-	2,37
Radial housing depth	tmin	(d4min-d3max)/2	0,41
	tmax	(d4max-d3min)/2	0,47
Diametrical stretch inside diameter O-ring installed (mm)	smin	d3min-d1max	0,00
	smax	d3max-d1min	0,07
Percentage of inside diameter stretch (%)	Smin	(smin/d1max)*100[%]	0,00
	Smax	(smax/d1min)*100[%]	3,02
Percentage of O-ring cross-sectional reduction resulting from diametric stretch (%)	Rmin	0,01 + 1,06(Smin) − 0,1 (Smin)2	0,00
	Rmax	0,01 + 1,06(Smax) − 0,1 (Smax)2	2,30
Cross section installed O-ring (mm)	d2*min	d2min –d2min(Rmax/100)	0,44
	d2*max	d2max –d2max(Rmin/100)	0,47
Percentage of effective O-ring cross-section compression (%)	Cmin	(d2*min-tmax)/d2min*	-5,76
	Cmax	(D2*max-tmin)/D2*max	12,77

Design for mockups with highly-compressed seal

Dimension	Symbol	Calculation	Min. and max. value including measurement accuracies
Inner diameter	d1	2,31	2,30
			2,32
Cross section	d2	0,56	0,55
			0,57
Piston diameter	d9min	3,05	3,04
	d9max	-	3,06
Bore diameter for piston application	d4min	3,2	3,19
	d4max	-	3,21
Housing inside diameter for piston application	d3min	2,4	2,39
	d3max	-	2,41
Radial housing depth	tmin	(d4min-d3max)/2	0,39
	tmax	(d4max-d3min)/2	0,41
Diametrical stretch inside diameter O-ring installed (mm)	smin	d3min-d1max	0,07
	smax	d3max-d1min	0,11
Percentage of inside diameter stretch (%)	Smin	(smin/d1max)*100[%]	3,02
	Smax	(smax/d1min)*100[%]	4,74
Percentage of O-ring cross-sectional reduction resulting from diametric stretch (%)	Rmin	0,01 + 1,06(Smin) − 0,1 (Smin)2	2,30
	Rmax	0,01 + 1,06(Smax) − 0,1 (Smax)2	2,79
Cross section installed O-ring (mm)	d2*min	d2min –d2min(Rmax/100)	0,53
	d2*max	d2max –d2max(Rmin/100)	0,56
Percentage of effective O-ring cross-section compression (%)	Cmin	(d2*min-tmax)/d2min*	23,32
	Cmax	(D2*max-tmin)/D2*max	29,97

Actual mockups with highly-compressed seal

Dimension	Symbol	Calculation	Min. and max. value including measurement accuracies
Inner diameter	d1	2,31	2,30
			2,32
Cross section	d2	0,56	0,55
			0,57
Piston diameter	d9min	-	2,96
	d9max	-	2,99
Bore diameter for piston application	d4min	-	3,19
	d4max	-	3,21
Housing inside diameter for piston application	d3min	-	2,28
	d3max	-	2,37
Radial housing depth	tmin	(d4min-d3max)/2	0,41
	tmax	(d4max-d3min)/2	0,47
Diametrical stretch inside diameter O-ring installed (mm)	smin	d3min-d1max	0,00
	smax	d3max-d1min	0,07
Percentage of inside diameter stretch (%)	Smin	(smin/d1max)*100[%]	0,00
	Smax	(smax/d1min)*100[%]	3,02
Percentage of O-ring cross-sectional reduction resulting from diametric stretch (%)	Rmin	0,01 + 1,06(Smin) − 0,1 (Smin)2	0,00
	Rmax	0,01 + 1,06(Smax) − 0,1 (Smax)2	2,30
Cross section installed O-ring (mm)	d2*min	d2min –d2min(Rmax/100)	0,54
	d2*max	d2max –d2max(Rmin/100)	0,57
Percentage of effective O-ring cross-section compression (%)	Cmin	(d2*min-tmax)/d2min*	13,47
	Cmax	(D2*max-tmin)/D2*max	28,07

Attachment 7 – Results of test runs

Inactive use with lowly-compressed seals

Results simulations runs with O-ring size 2,31x0,46 – inactive use conditions						
Experiment	**Block**	**Axle**	**Total time**	**Pass leakage test**	**Pressure leak**	**#CFU**
14	1	3	60	No	0-90	full
14	2	2	60	Yes	>300	0
15	2	3	61	No	0-90	full
15	3	1	61	No	0-90	full
15	1	2	61	No	0-90	full
16	3	2	59	No	0-90	>300
16	1	3	60	No	0-90	full
16	2	1	61	Yes	181-299	0
17	2	2	59	Yes	>300	0
17	3	3	59	Yes	>300	0
17	1	1	60	No	0-90	full
18	3	1	60	No	0-90	full
18	1	2	60	No	0-90	>300
18	2	3	60	No	0-90	full
19	1	3	62	Yes	>300	0
19	2	1	62	No	0-90	full
19	3	2	63	No	0-90	0
20	3	3	62	Yes	181-299	0
20	1	1	63	No	0-90	full
20	2	2	63	Yes	>300	0
21	1	2	60	No	91-180	0
21	2	3	60	Yes	>300	0
21	3	1	61	No	0-90	full
22	2	1	67	No	0-90	full
22	3	2	68	No	0-90	>300
22	1	3	68	No	0-90	full
23	1	1	64	No	0-90	full
23	2	2	64	Yes	>300	0
23	3	3	64	No	0-90	full

Experiment	Block	Axle	Total time	Pass leakage test	Pressure leak	#CFU	Type of control
14	Control	None	60	Yes	>300	0	immersion
16	Control	None	59	Yes	>300	0	immersion
excluded (not being K.Pneumonia,according to MALDI-TOF)							
None							

CFU of leakage passing tests - static			Bacterial penetration?		
0	9	100%	yes	0%	0
1 till 49	0	0%	no	100%	9
>50	0	0%			
>100	0	0%	Mean time±SD		
>300	0	0%	61,7931	2,5267047	
Full	0	0%			

Inactive use with highly-compressed seals

Results simulations runs with O-ring size 2,31x0,56 – inactive use conditions						
Experiment	Block	Axle	Total time	Pass leakage test	Pressure leak	#CFU
1	2	2	60	No	91-180	full
1	3	3	60	Yes	>300	0
2	2	3	60	Yes	>300	0
2	3	1	62	Yes	>300	0
2	1	2	63	Yes	181-299	0
3	3	2	60	Yes	>300	0
3	1	3	60	Yes	>300	0
3	2	1	61	Yes	>300	1 till 49
5	3	1	61	Yes	>300	0
5	1	2	62	Yes	>300	0
5	2	3	62	Yes	>300	0

Experiment	Block	Axle	Total time	Pass leakage test	Pressure leak	#CFU	Type of control
3.1	Control	1	60	Yes	>300	0	immersion
5-0	Control	None	63	Yes	>300	0	solid block
excluded (not being K.Pneumonia,according to MALDI-TOF)							
4-do not include	2	2	60	Yes	>300	>100	not K. Pneum
4-do not include	3	3	0	Yes	>300	1 till 49	not K. Pneum
4-do not include	1	1	61	No	0-90	full	not K. Pneum

CFU of leakage passing tests - Passive conditions (n=10)			Bacterial penetration?		
0	9	90%	yes	10%	1
1 till 49	1	10%	no	90%	9
>50	0	0%			
>100	0	0%	Mean time±SD		
>300	0	0%	61	1,095445	
Full	0	0%			

Pressure on dirty side with highly-compressed seals

Results simulations runs with O-ring size 2,31x0,56 – pressure						
Experiment	**Block**	**Axle**	**Total time**	**Pass leakage test**	**Pressure leak**	**#CFU**
7	1	1	61	No	0-90	0
7	2	2	60	Yes	>300	0
7	3	3	59	Yes	>300	0
8	2	1	60	Yes	>300	0
8	3	2	60	Yes	>300	0
8	1	3	61	Yes	>300	0
9	1	3	63	Yes	>300	0
10	2	2	57	Yes	>300	0
10	1	1	61	Yes	>300	full
13	3	1	60	Yes	>300	0
13	1	2	61	Yes	>300	0
13	2	3	58	Yes	>300	0

Experiment	**Block**	**Axle**	**Total time**	**Pass leakage test**	**Pressure leak**	**#CFU**	**Type of control**
7-0	**Control**	**None**	**60**	**Yes**	**>300**	**0**	**solid block**
excluded (not being K.Pneumonia,according to MALDI-TOF)							
9-not not include	**3**	**2**	**61**	**Yes**	**>300**	**full**	**not K. Pneum**
9- no not include	**2**	**1**	**63**	**Yes**	**>300**	**full**	**not K. Pneum**
10-do not include	**3**	**3**	**59**	**Yes**	**>300**	**full**	**not K. Pneum**

CFU of leakage passing tests - External pressure (n=11)			**Bacterial penetration?**		
0	**10**	**91%**	**yes**	**9%**	**1**
1 till 49	**0**	**0%**	**no**	**91%**	**10**
>50	**0**	**0%**			
>100	**0**	**0%**	**Mean time**	**±SD**	
>300	**0**	**0%**	**60,08333**	**1,5642793**	
Full	**1**	**9%**			

Rotating axle with highly-compressed seals

Results simulations runs with O-ring size 2,31x0,56 – rotation						
Experiment	**Block**	**Axle**	**Total time**	**Pass leakage test**	**Pressure leak**	**#CFU**
11	1	1	58	Yes	>300	full
11	2	2	58	Yes	>300	1 till 49
11	3	3	58	Yes	>300	0
6	1	1	61	Yes	>300	full
14	1	2		Yes	>300	full
14	2	3		Yes	>300	full
14	3	1		Yes	>300	0
15	1	3		Yes	>300	full
15	2	1		Yes	>300	0
15	3	2		Yes	>300	0

Experiment	Block	Axle	Total time	Pass leakage test	Pressure leak	#CFU	Type of control
12	2	3	60	Yes	>300	0	broth both
12	3	1	60	Yes	>300	0	broth both
12	1	2	61	Yes	>300	0	broth both
excluded (not being K.Pneumonia,according to MALDI-TOF)							
6	3	3	60	Yes	>300	full	leak
6	2	2	61	Yes	>300	full	pump

CFU of leakage passing tests - Rotation axle (n=10)			Bacterial penetration?		
0	4	40%	yes	60%	6
1 till 49	1	10%	no	40%	4
>50	0	0%			
>100	0	0%	Mean time±SD		
>300	0	0%	58,75	1,5	
Full	5	50%			

Rotating, translating axle with highly-compressed seals

Results simulations runs with O-ring size 2,31x0,56 - passive conditions						
Experiment	**Block**	**Axle**	**Total time**	**Pass leakage test**	**Pressure leak**	**#CFU**
16	1	1	60	Yes	>300	1 till 49
16	2	2	60	Yes	>300	full
16	3	3	61	Yes	>300	0
17	2	3	60	Yes	>300	0
17	3	1	60	Yes	>300	>100
17	1	2	60	Yes	>300	full
18	3	2	60	Yes	>300	0
18	1	3	61	Yes	>300	full
18	2	1	61	Yes	>300	full

Experiment	**Block**	**Axle**	**Total time**	**Pass leakage test**	**Pressure leak**	**#CFU**
18	Control	None	60	Yes	>300	0
17	Control	None	80	Yes	>300	0
12	1	2	61	Yes	>300	0
excluded (not being K.Pneumonia,according to MALDI-TOF)						
None						

CFU of leakage passing tests Rotation axle with axial translation (n=9)			Bacterial penetration?		
0	3	33%	yes	67%	6
1 till 49	1	11%	no	33%	3
>50	0	0%			
>100	1	11%	Mean time±SD		
>300	0	0%	60,33333	0,5	
Full	4	44%			

Attachment 8 – Statistical tests

First set (1st and 2nd H0)

The first set of statistical tests was performed to analyze whether air- and bacterial tightness ('air/bacterial penetration' = 1 and 'air/bacterial tight' = 2) and effective compression ('low' = 1 and 'high' = 2) are independent of one another.

1st H0 – Air tightness and the effective compression are independent

Pearson Chi-Square test 1- H1					
R, c	**$E_{r,c}$**	**$O_{r,c}$**	**(O-E)^2/E**	**χ^2-Value**	**Critical χ^2-Value**
Loose seal – pass (1,1)	**13,775**	**9**	**1,6552178**	**11,464712**	**Lower 0,004; Upper 3,841**
Loose seal – fail (1,2)	**15,225**	**20**	**1,497578**		
Tight seal – pass (2,1)	**5,225**	**10**	**4,363756**		
tight seal – fail (2,2)	**5,775**	**1**	**3,9481602**		

2nd H0 – Bacterial tightness and effective compression are independent

Pearson Chi-Square test 1 – H2					
R, c	**$E_{r,c}$**	**$O_{r,c}$**	**(O-E)^2/E**	**χ^2-Value**	**Critical χ^2-Value**
Loose seal – no microbial leakage (1,1)	**8,5263158**	**9**	**0,0263158**	**0,95**	**Lower 0,004; Upper 3,841**
Loose seal – microbial leakage (1,2)	**0,4736842**	**0**	**0,4736842**		
Tight seal – no microbial leakage (2,1)	**9,4736842**	**9**	**0,0236842**		
Tight seal – microbial leakage (2,2)	**0,5263158**	**1**	**0,4263158**		

Second set (3rd, 4th and 5th H0)

The second set of statistical tests were performed to analyze whether bacterial tightness ('bacterial penetration' = 1 and 'bacterial tight' = 2) and usage variables ('variables zero'= 1 and 'variable as in use = 2) are independent of one another.

3rd H0 – Bacterial tightness and gauge pressure on the dirty side are independent

Pearson Chi-Square test - H3					
R, c	**$E_{r,c}$**	**$O_{r,c}$**	**(O-E)^2/E**	**χ^2-Value**	**Critical χ^2-Value**
1,1	**9,047619**	**9**	**0,0002506**	**0,0050239**	**Lower 0,004; Upper 3,841**
1,2	**0,952381**	**1**	**0,002381**		
2,1	**9,952381**	**10**	**0,0002278**		
2,2	**1,047619**	**1**	**0,0021645**		

4th H0 – Bacterial tightness and dynamically rotating loading of the mockups' axles are independent

Pearson Chi-Square test 2 - H4					
R, c	$E_{r,c}$	$O_{r,c}$	(O-E)^2/E	χ^2-Value	Critical χ^2-Value
1,1	6,5	9	0,9615385	5,4945055	Lower 0,004; Upper 3,841
1,2	3,5	1	1,7857143		
2,1	6,5	4	0,9615385		
2,2	3,5	6	1,7857143		

5th H0 – Bacterial tightness and dynamically rotating loading with axial displacement of the mockups' axles are independent

Pearson Chi-Square test 2 - H5					
R, c	$E_{r,c}$	$O_{r,c}$	(O-E)^2/E	χ^2-Value	Critical χ^2-Value
1,1	6,3157895	9	1,1407895	6,5369048	Lower 0,004; Upper 3,841
1,2	3,6842105	1	1,9556391		
2,1	5,6842105	3	1,2675439		
2,2	3,3157895	6	2,1729323		

Third set

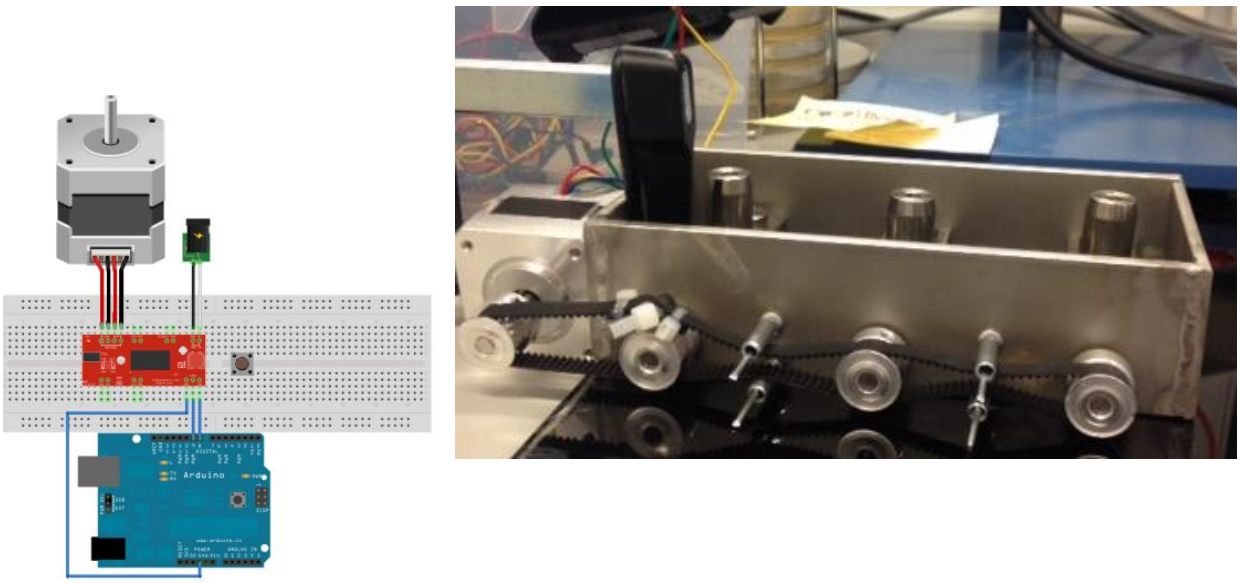
A statistical test was performed to analyze whether bacterial tightness ('bacterial penetration' = 1 and 'bacterial tight' = 2) and effective compression ('low' = 1 and 'high' = 2) are independent of one another.

Pearson Chi-Square test 3					
R, c	$E_{r,c}$	$O_{r,c}$	(O-E)^2/E	χ^2-Value	Critical χ^2-Value
1,1	3,6842105	4	0,0270677	0,0904762	Lower 0,004; Upper 3,841
1,2	6,3157895	6	0,0157895		
2,1	3,3157895	3	0,0300752		
2,2	5,6842105	6	0,0175439		

Attachment 9 - Stepper motor feature to automate test runs of the controlled investigation

The stepper motor installation system was adapted to the mock-up system for the controlled-investigation with the intention to use it for automatic dynamic rotational loading of the axle to closely simulate use of the elevator lever system. The stepper motor was not used in the experiment, since manual manipulations was possible, while reducing the back lash in the automated system was time consuming. For future larger-scaled experiments, such a stepper motor can be implemented, to save time and effort.

The stepper motor installation was a stepper motor (Sparkfun) controlled by a stepper motor driver (Easydriver) and Arduino board (Uno Rev3). The installation was done during jacks. (a) The Arduino code gave the stepper motor an output of turning 90° back-and-forth for 30 times over a total period of 60 minutes by repeating the same cycle every 2 minutes; a 1 second movement of 90° forth, is followed after 1 minute break by a 90° backwards 1 second movement. The stepper motor and the axles of the experimental construction were geared by four identical aluminum T5 pulleys. The stepper motor and the axles were connected by a T5 timing belt (Reprapworld.com) while tensioned in the bearing system. (Attachment8-Figure 1b)

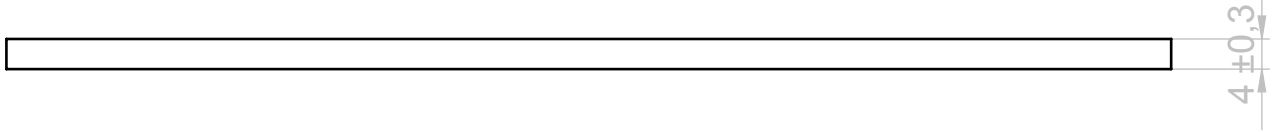


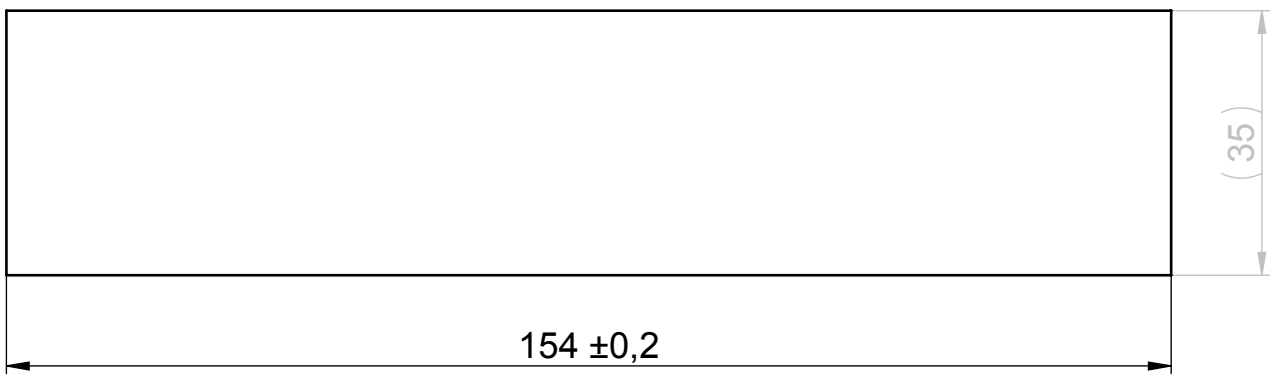
Attachment8-Figure 1(left) and (right)b. a: The stepper motor installation scheme. Adapted from [66] b: the stepper motor adapter to the mock-up construction

```
#include <Stepper.h>
```

```
const int stepsPerRevolution = (200 * 32) ;  // steps per full revolution
const int spd_movement = 1; // time (seconds) of the 90-degrees movement
const int spd = 60/ (4 * spd_movement); // RPM

// initialize the stepper library on pins 8 through 11:
Stepper myStepper(stepsPerRevolution, 8, 9);

void setup() {
  pinMode(2, INPUT_PULLUP);
  // set the speed:
  myStepper.setSpeed(spd);
  // initialize the serial port:
  Serial.begin(9600);
   while (digitalRead(2) == HIGH) {
    delay(100);
  }
}

void loop() {
  // 90-degrees movement in one direction:
  Serial.println("clockwise");
  myStepper.step(stepsPerRevolution / 4);
  delay(60000-1000); //no movement for 59 seconds

  // 90-degrees movement in the other direction:
  Serial.println("counterclockwise");
  myStepper.step(-stepsPerRevolution / 4 );
  delay(60000-1000); //no movement for 59 seconds
}
```

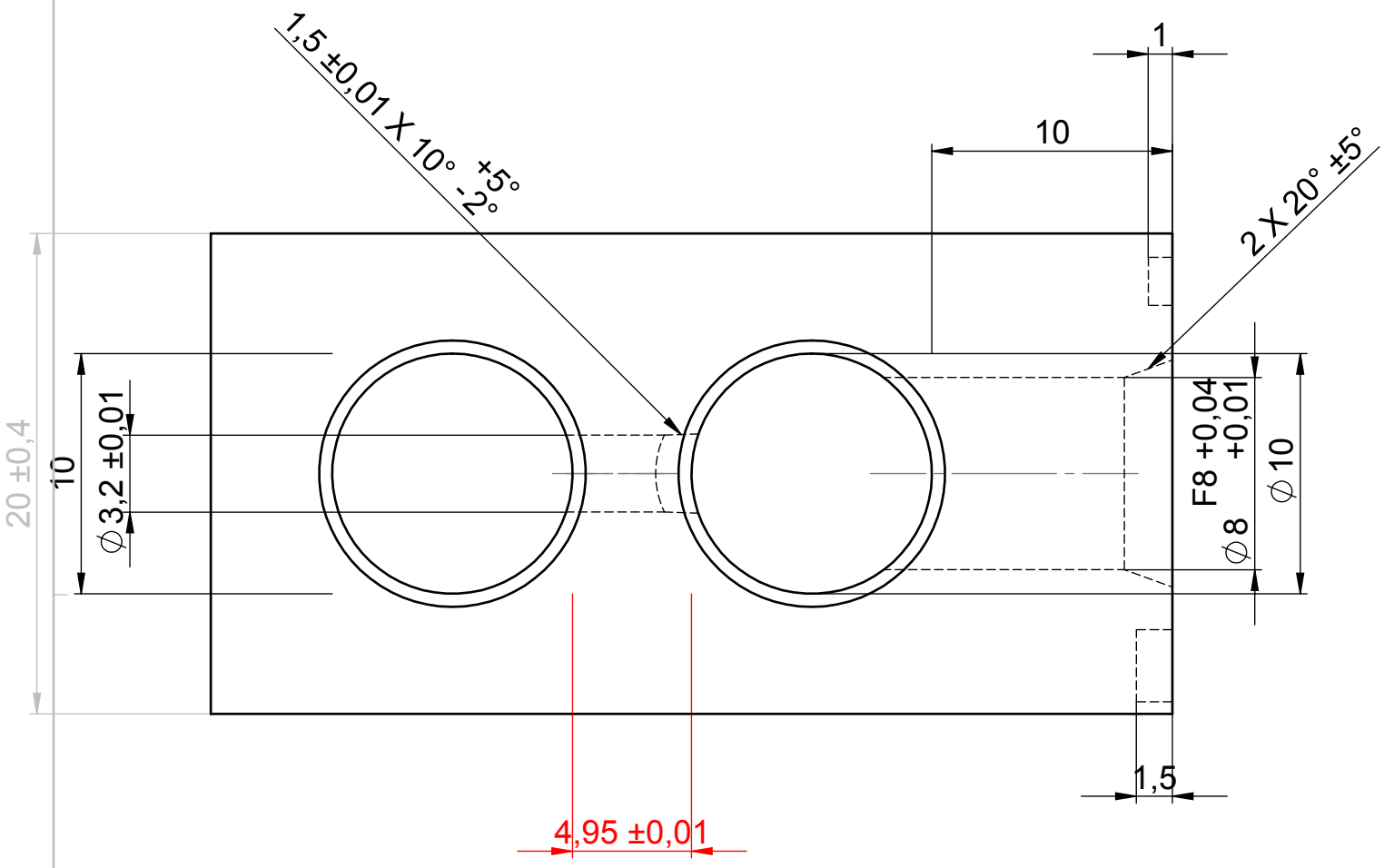
Attachment 10 - Construction drawings of the experiment setup

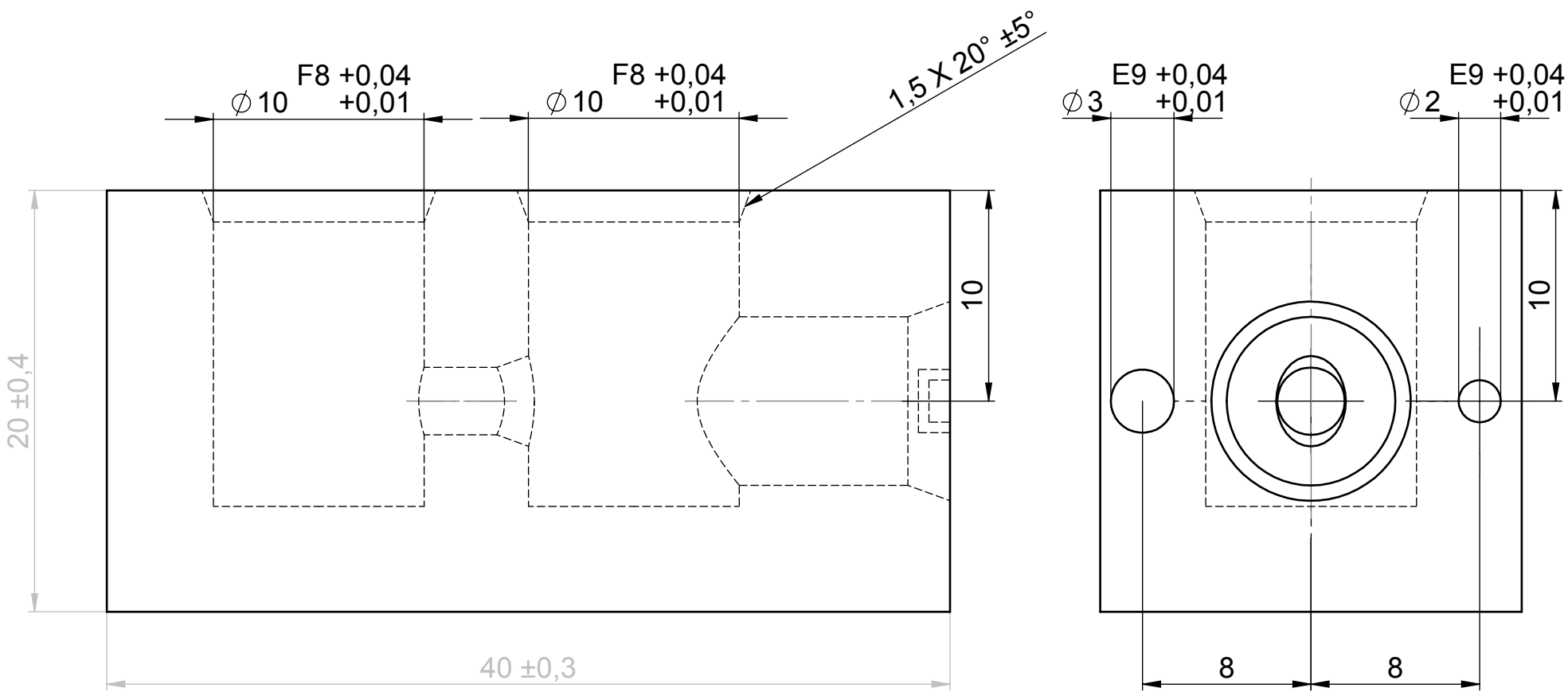


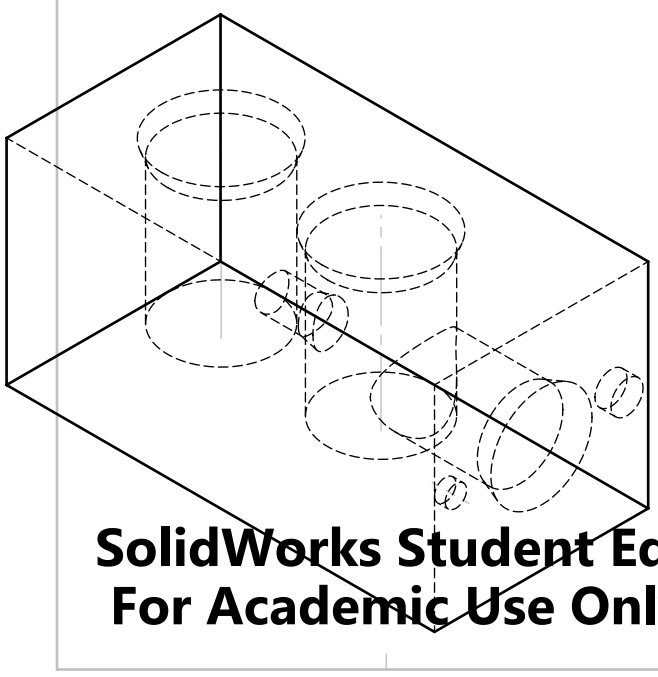


schaal 1:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP			gewicht gram
benaming drawning_construction3__backplate			
TU Delft Industrial Design Engineering		formaat A4	tekeningnummer 1

Mat.: AISI 1035 Steel (SS)
Aantal: 3







schaal 4:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht 102 gram	
benaming drawning_construction3__block_v1			

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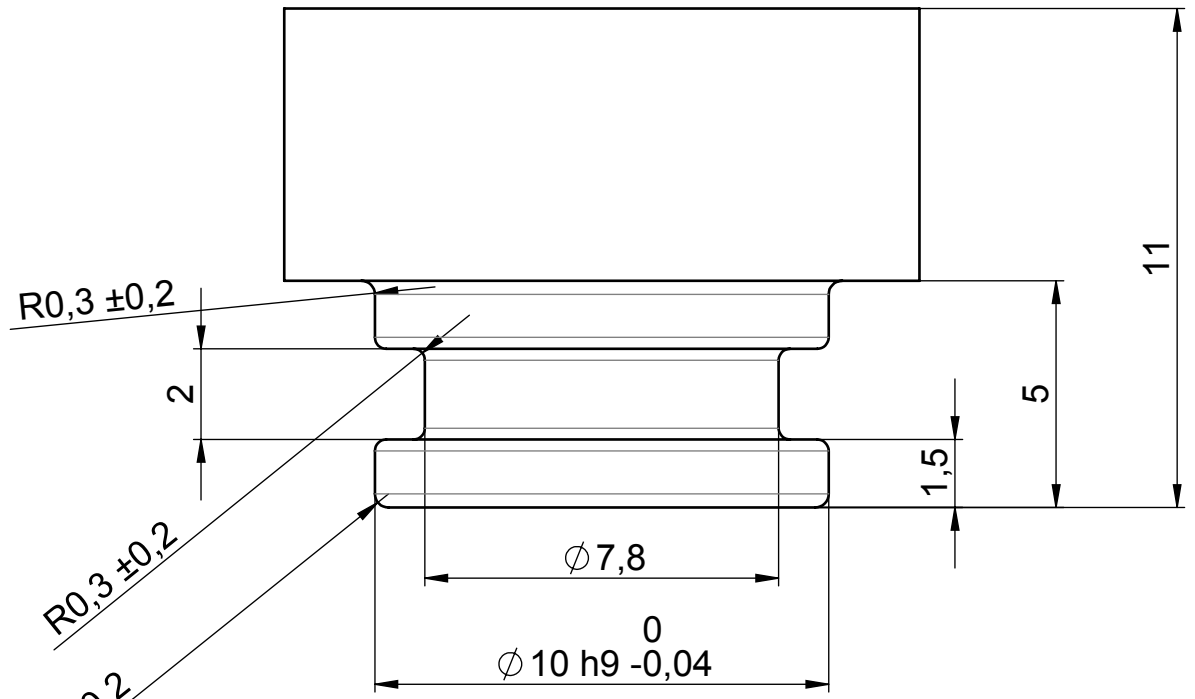
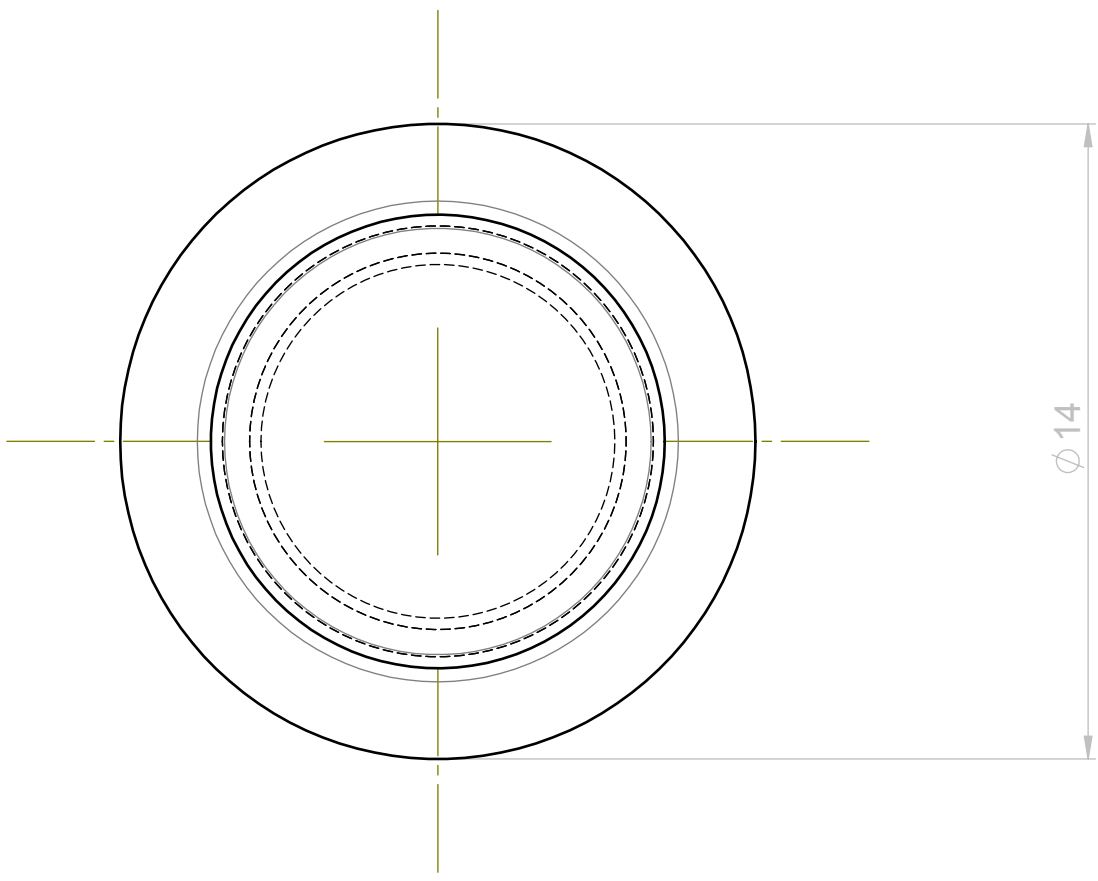
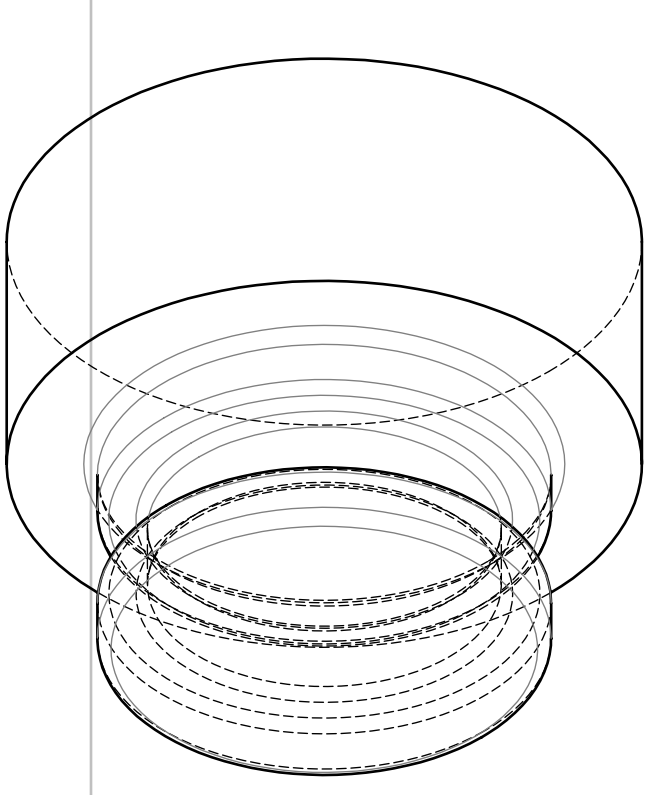
Mat.: RVS
Aantal: << 3>>







schaal 4:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht 10 gram	

benaming drawning_construction3__cover

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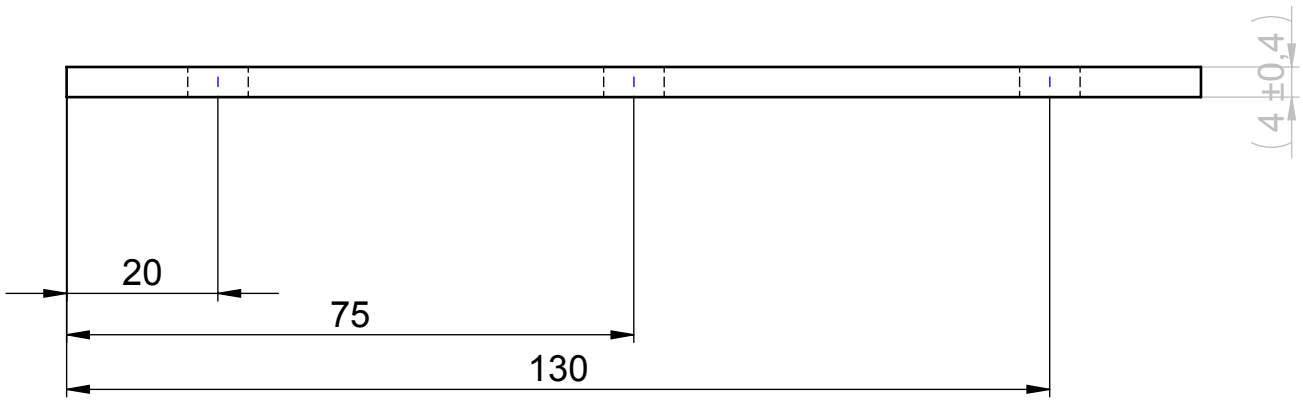

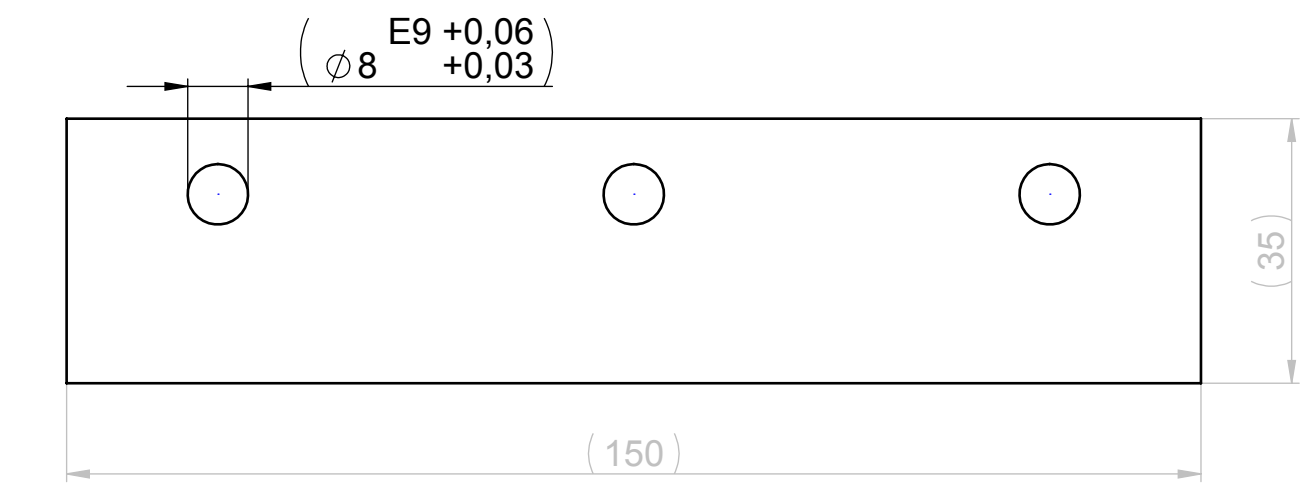

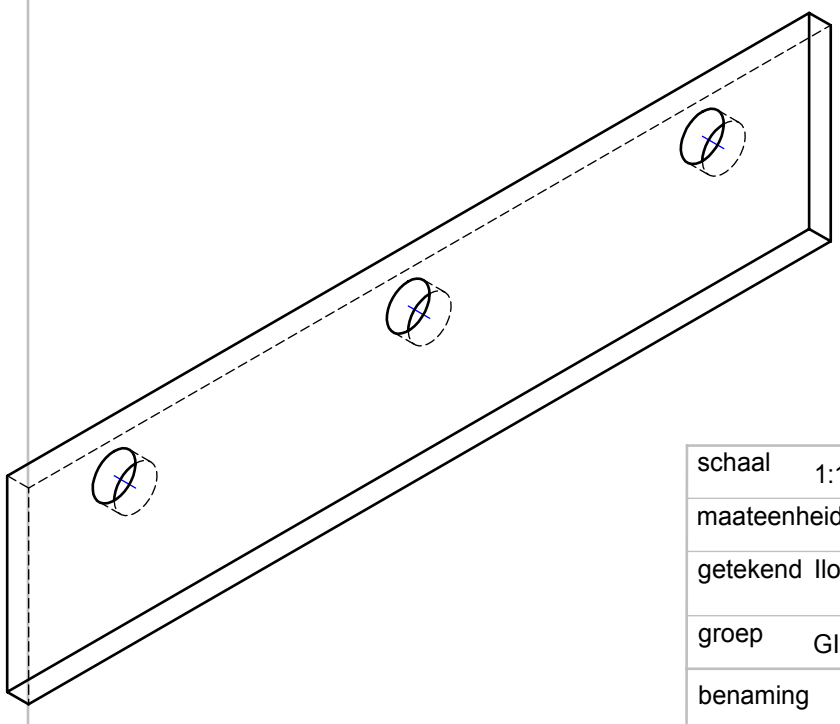

schaal 1:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht	gram

benaming drawning_construction3__frontplate

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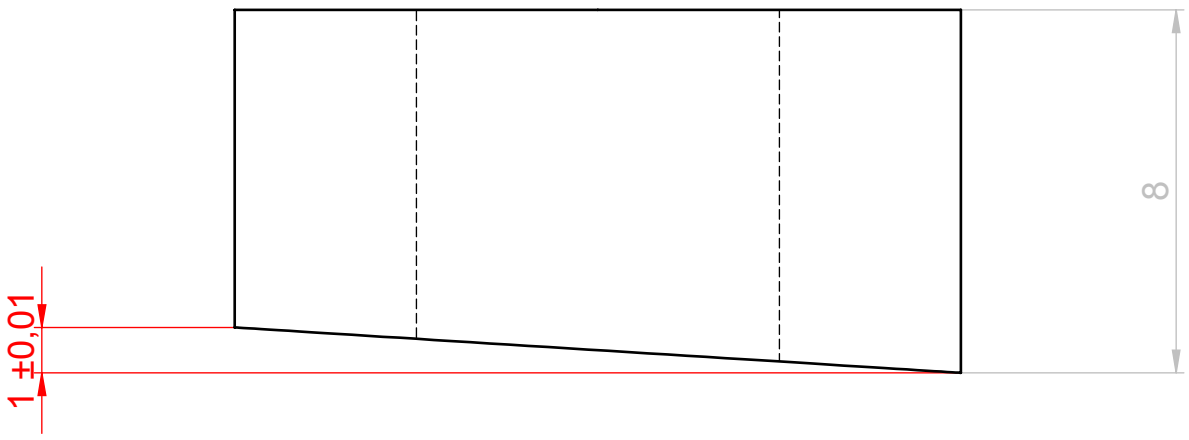

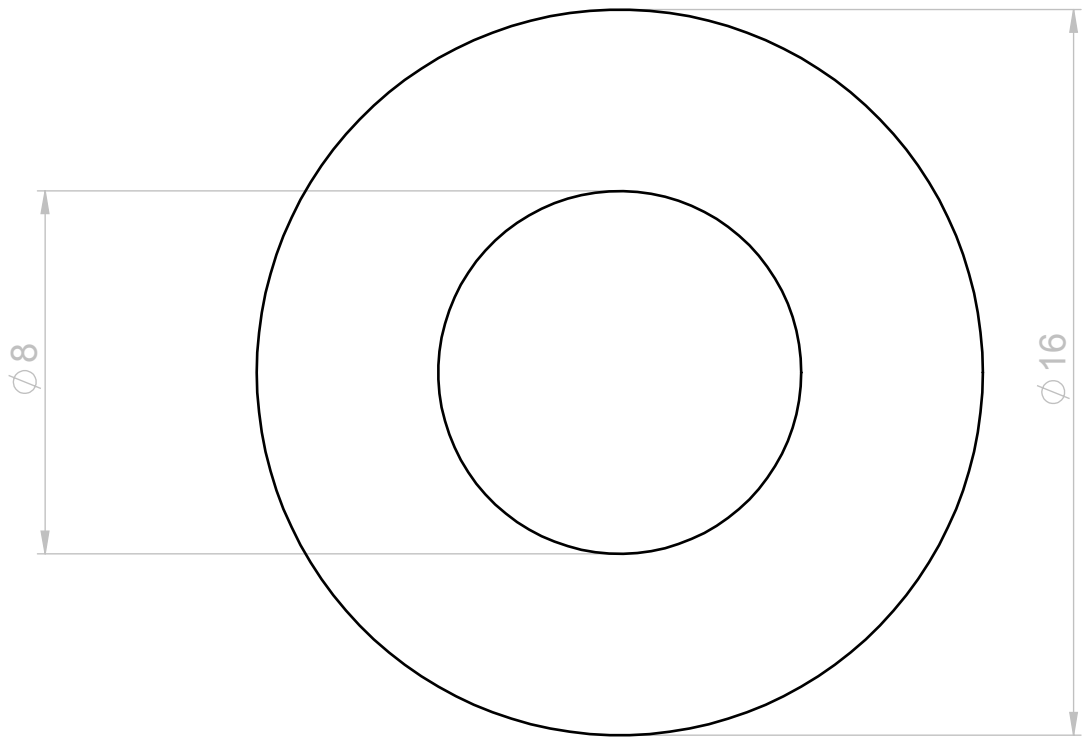

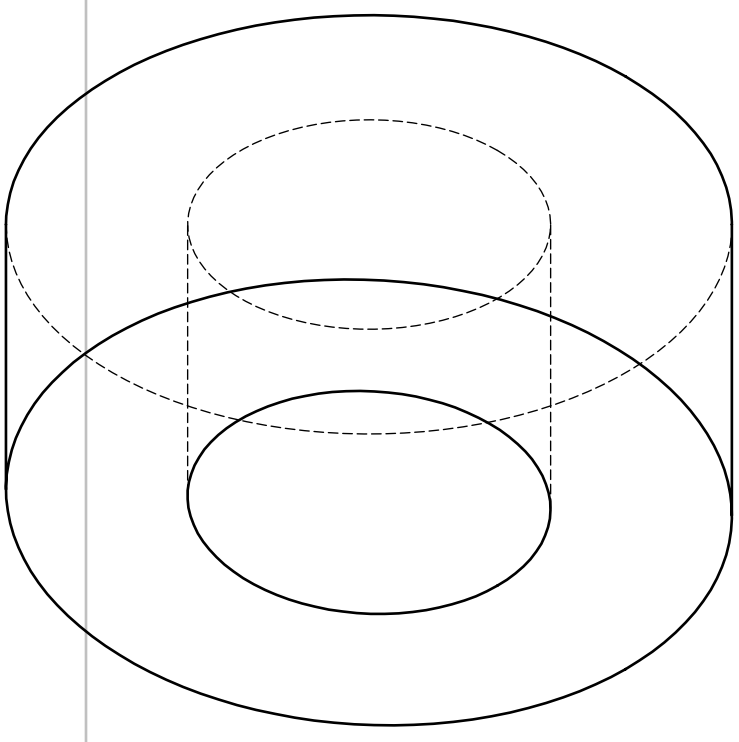

schaal 6:1		datum 19-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht	gram

benaming drawning_construction3__lock

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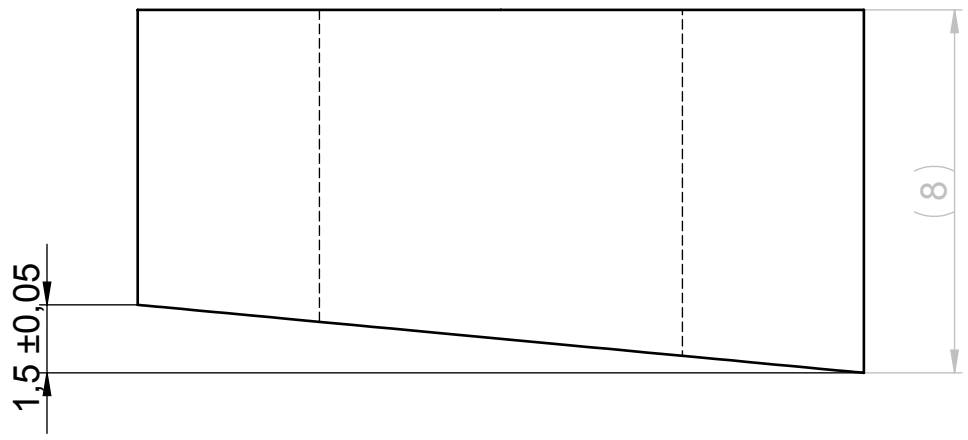

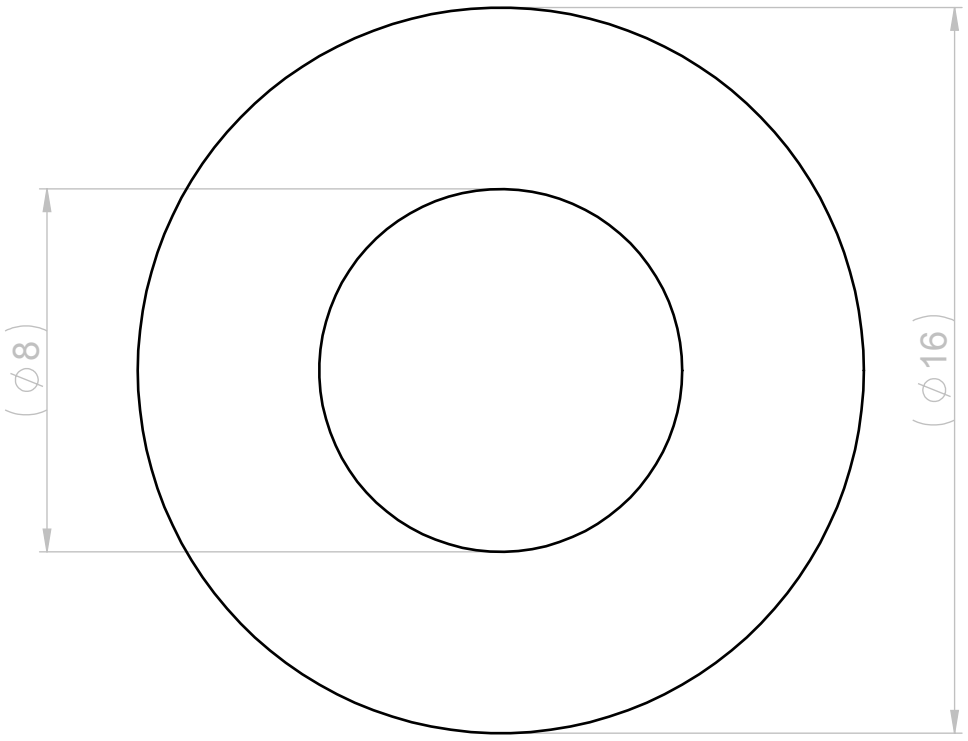

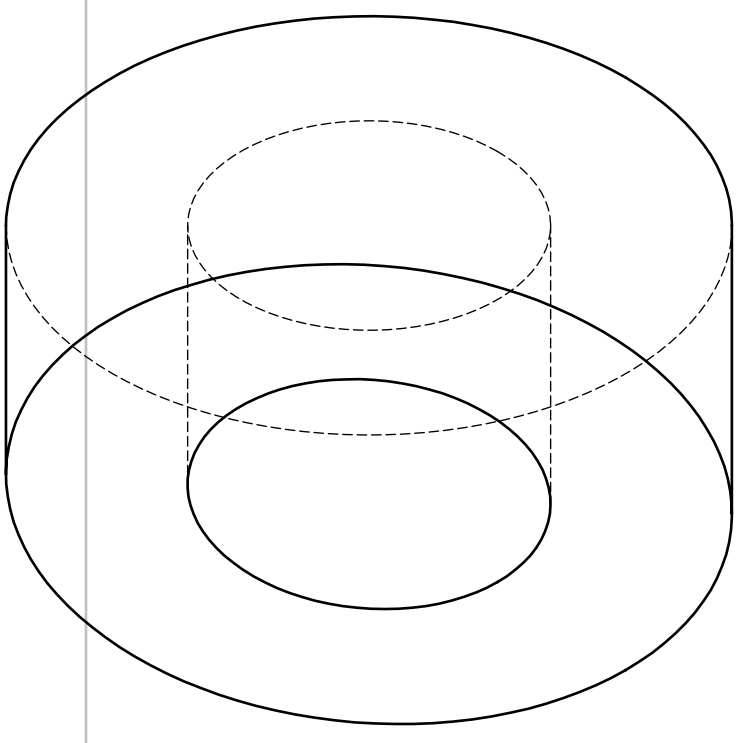

schaal 6:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht	gram

benaming drawning_construction3__lock1.5mm

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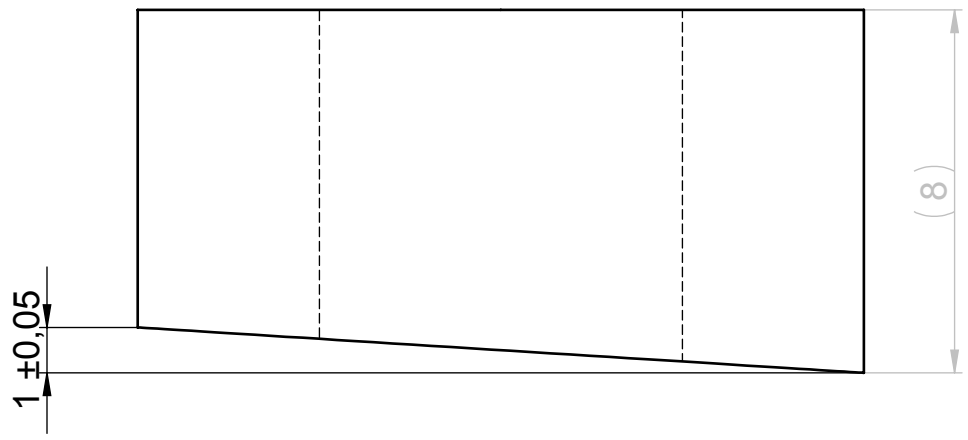

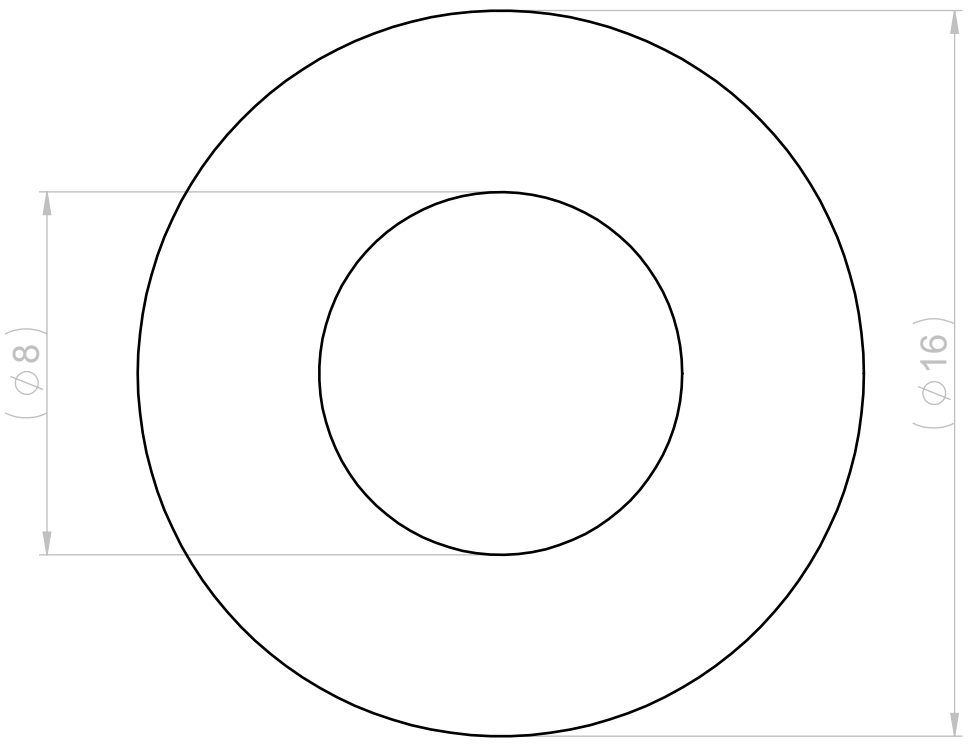

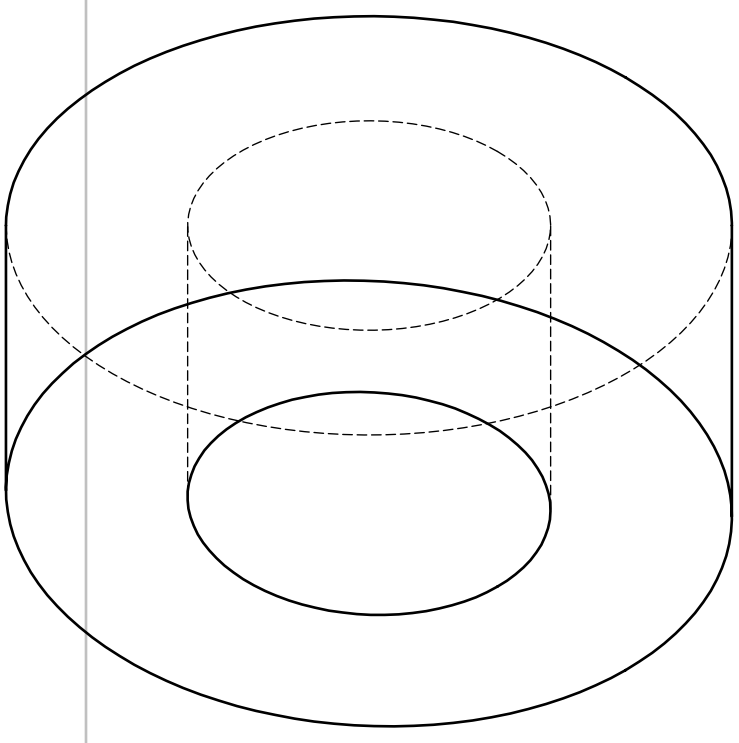

schaal 6:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht	gram
benaming drawning_construction3__lock1mm			
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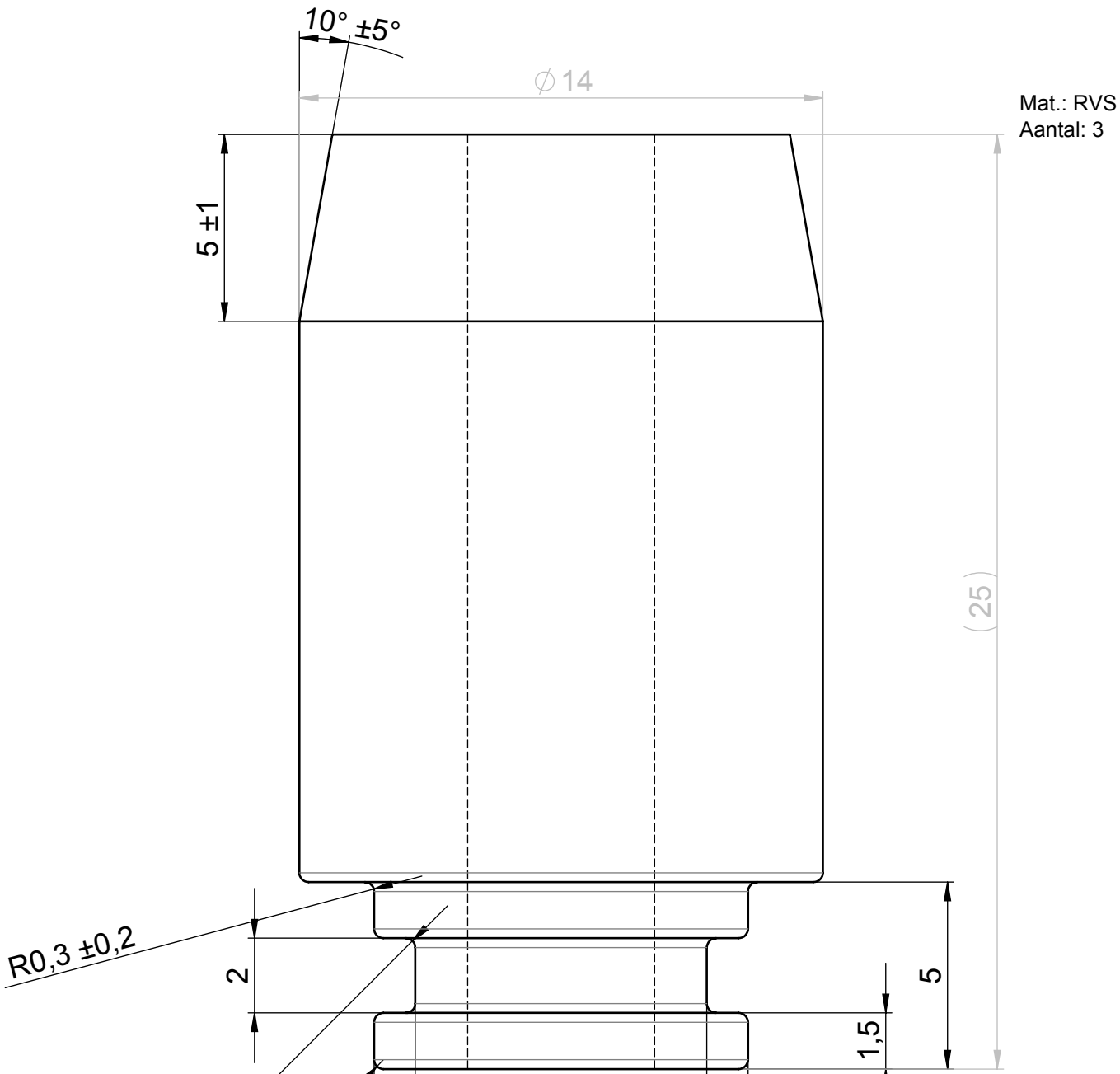

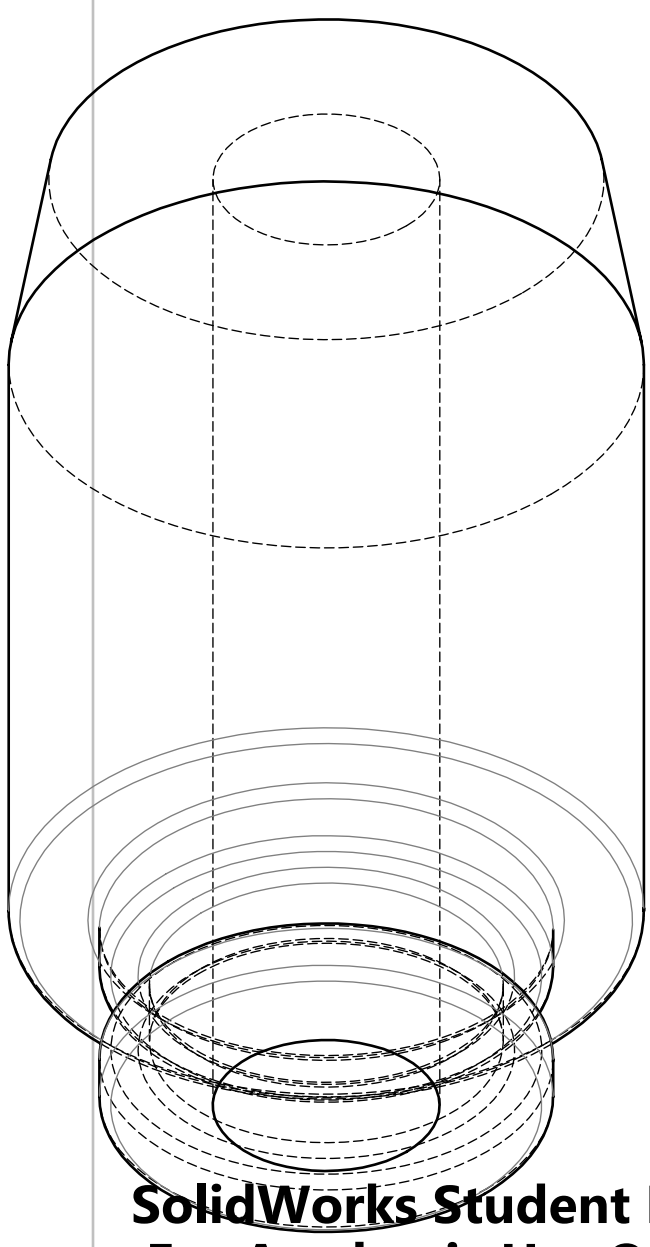

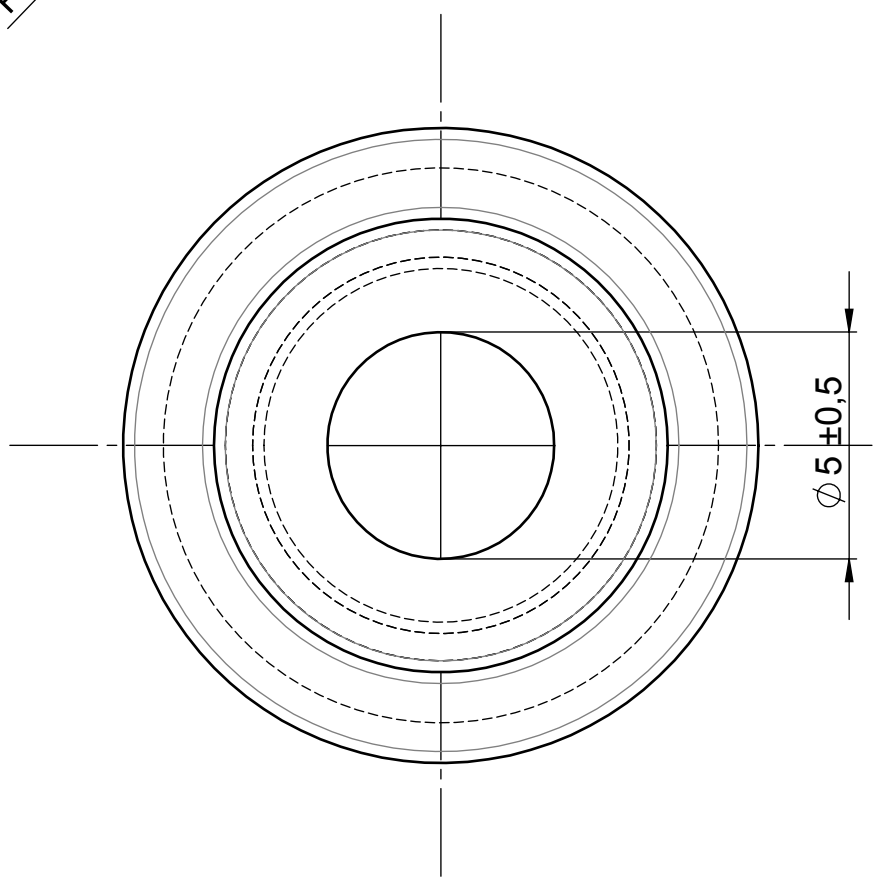

schaal 4:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht 23 gram	
benaming drawning_construction3__pump			
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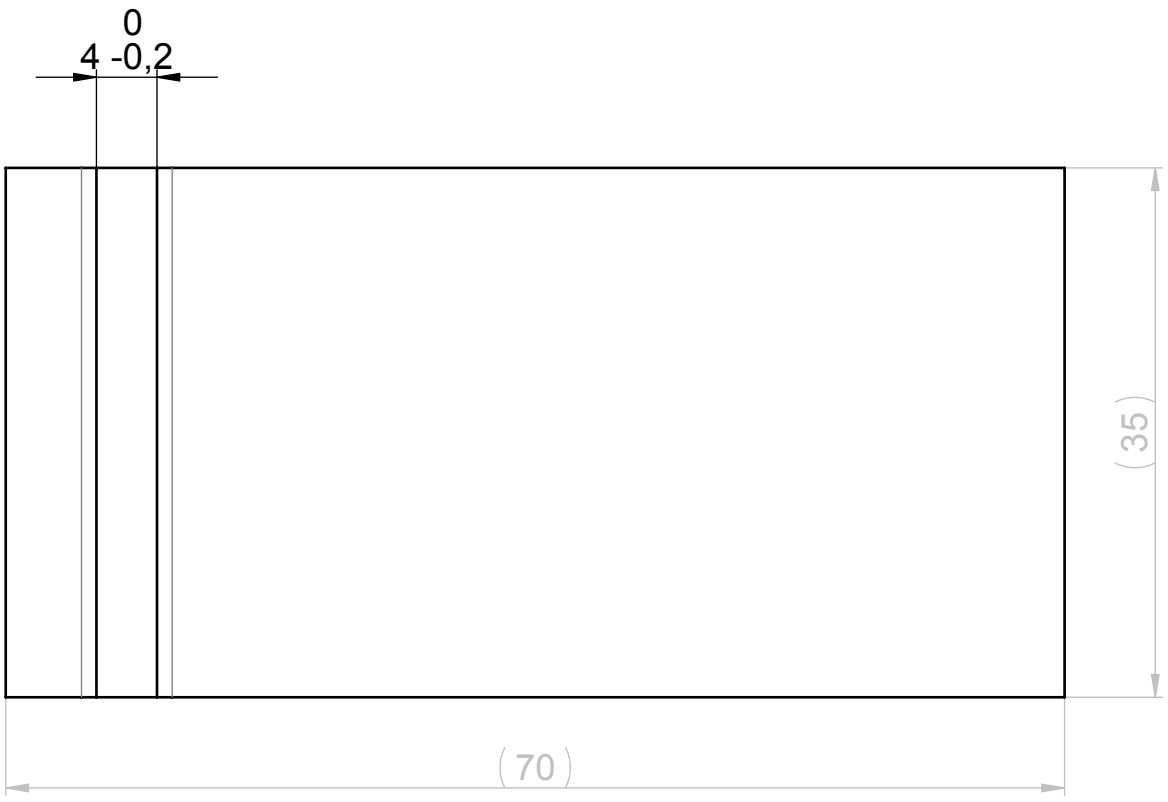

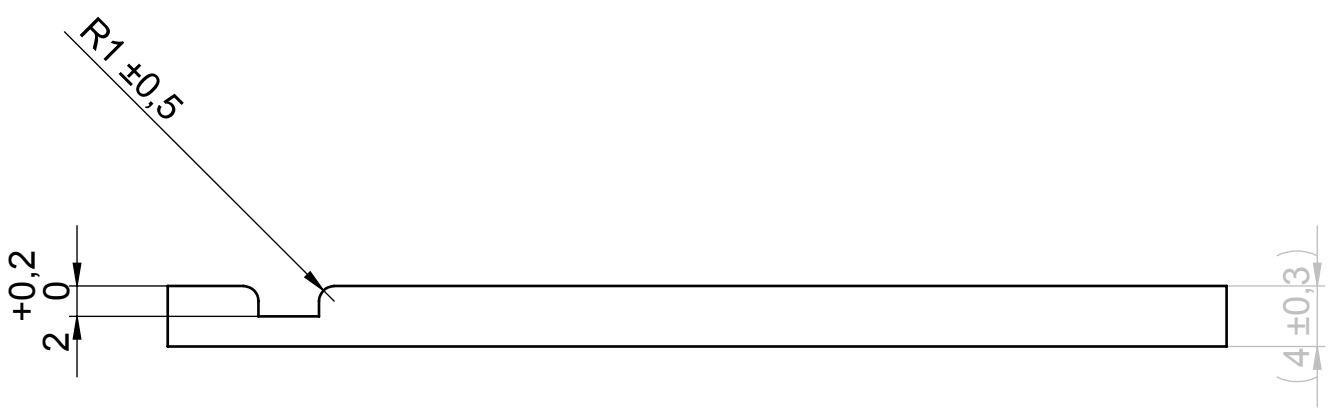

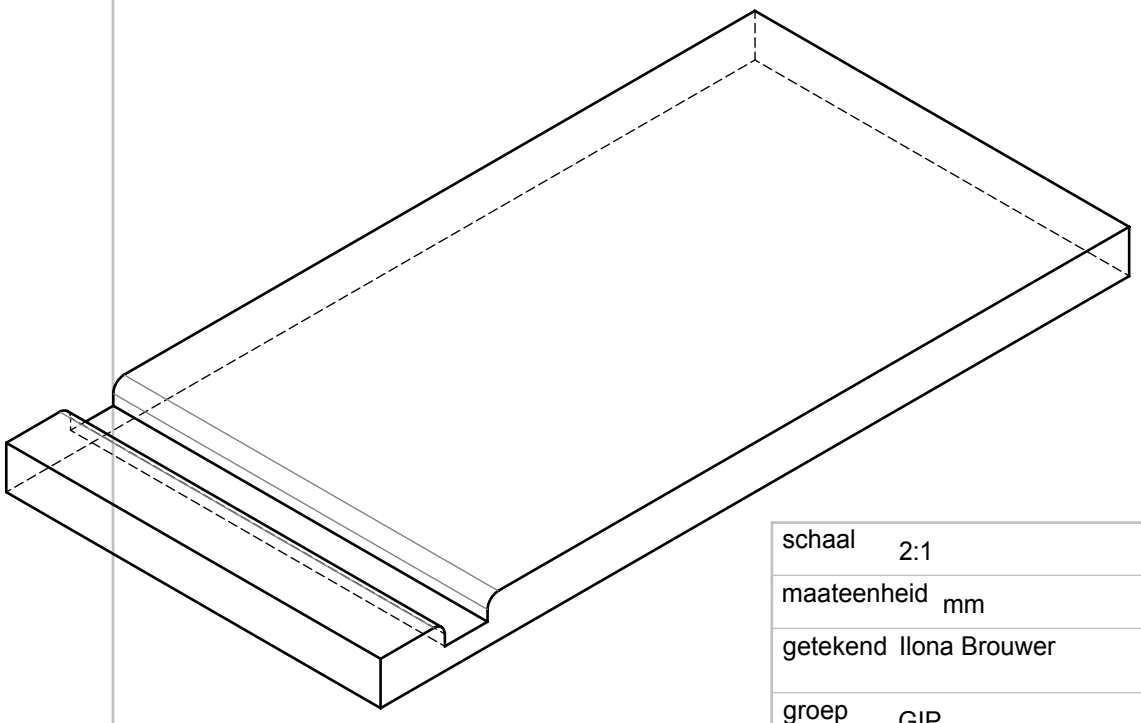

schaal 2:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP			gewicht gram
benaming drawning_construction3__sideplate			

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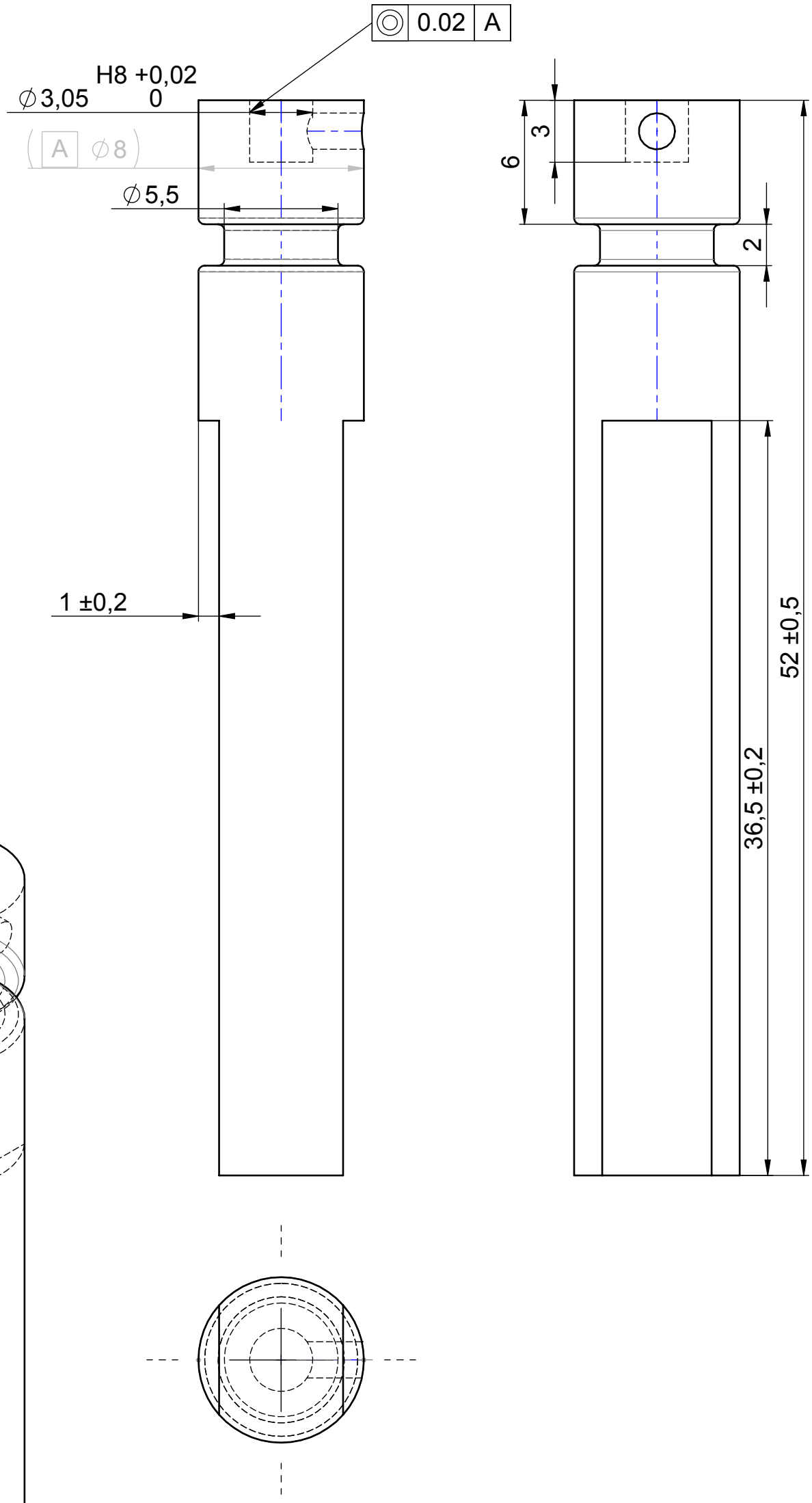
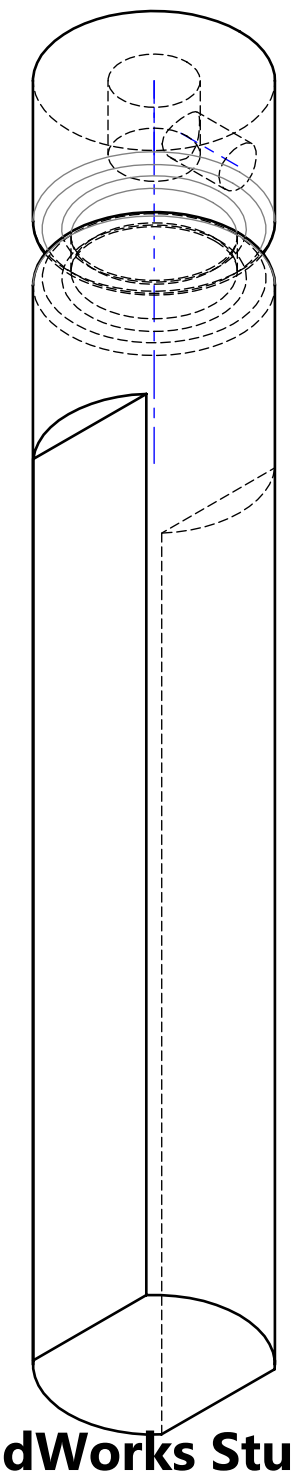
Mat.: RVS
Aantal:3





schaal 4:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht 18 gram	

benaming drawning_construction3__thickaxle

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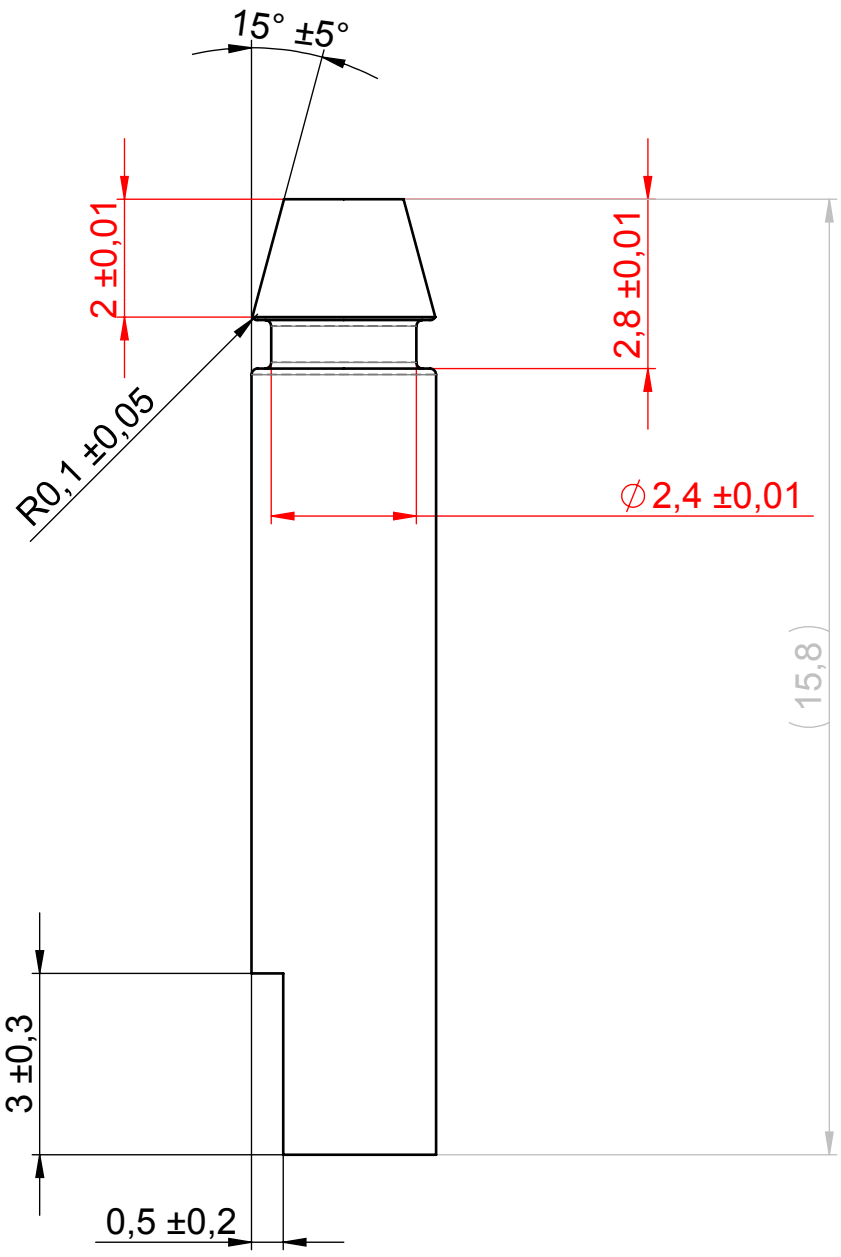
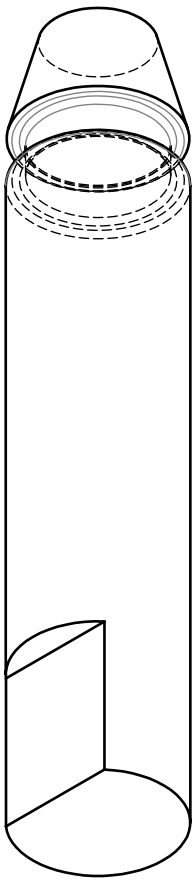
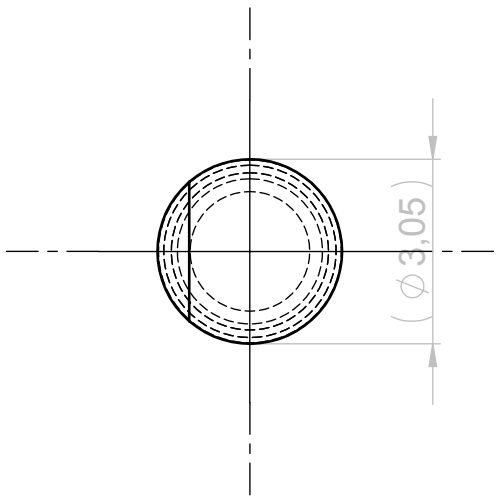
Mat.: AISI 1035 Steel (SS)
Aantal: 3







schaal 8:1		datum 20-4-2016	opmerkingen Toleranties gelden de standaardtolerantie IT10 (volgens NEN ISO 286-1), tenzij anders aangegeven
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht 1 gram	

benaming drawning_construction3__thinaxle

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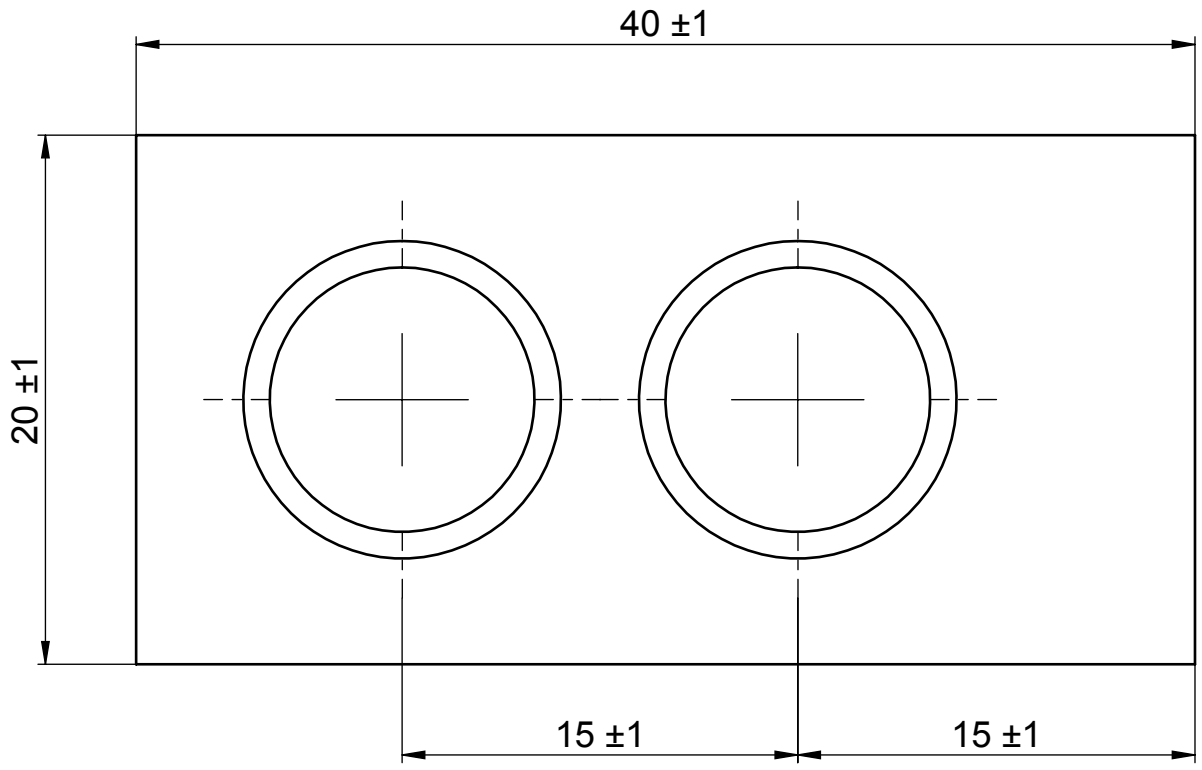
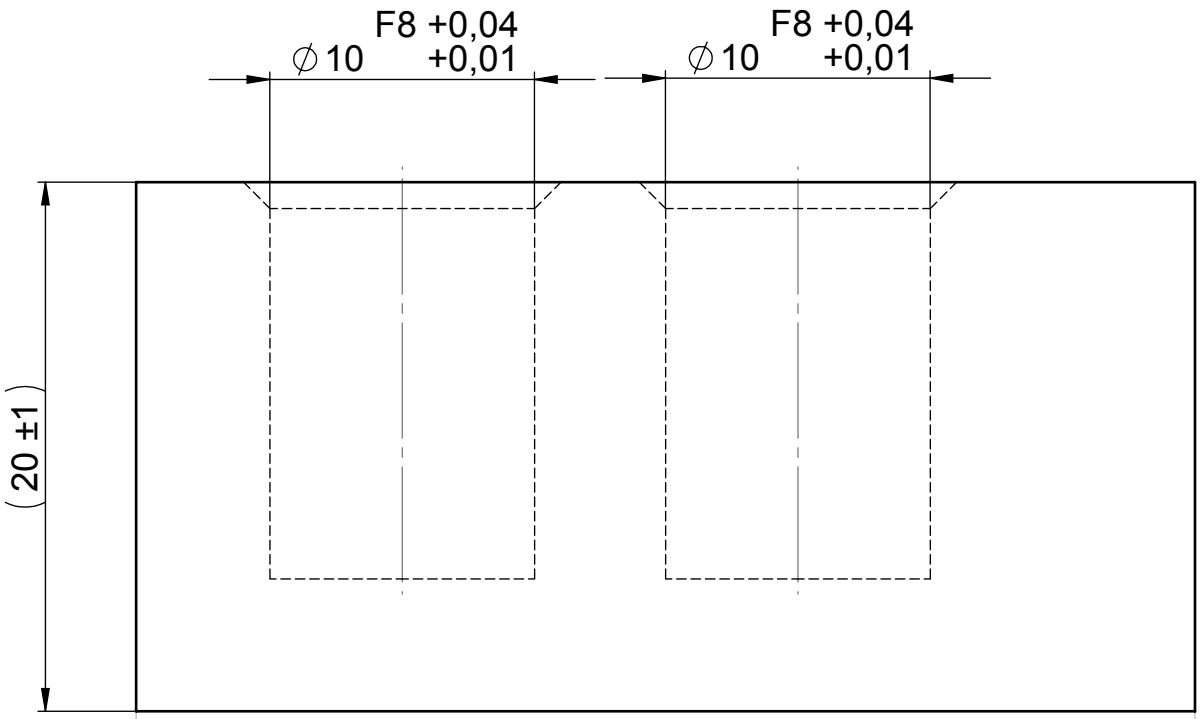
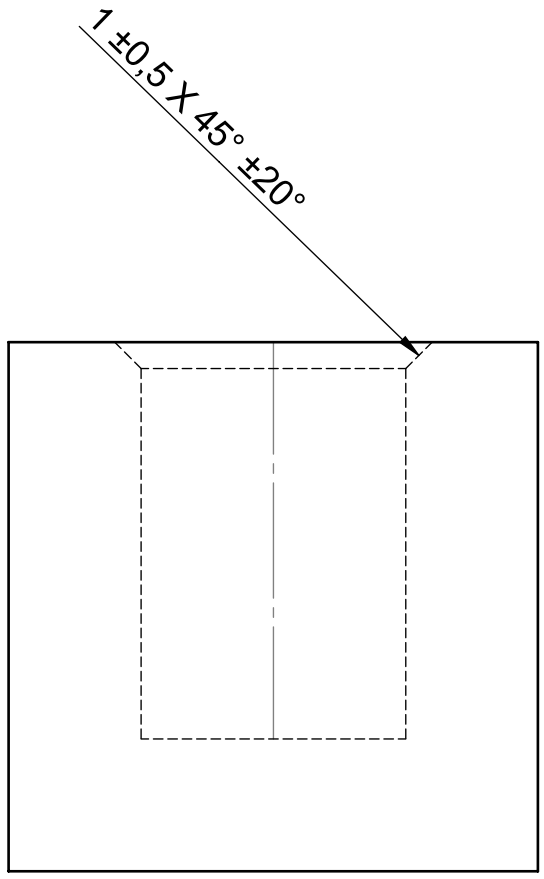
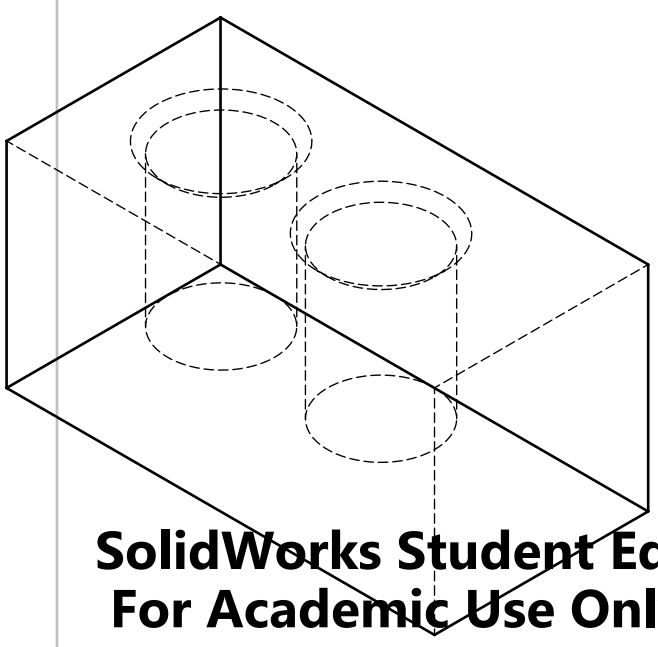
Mat.: AISI 1035 Steel (SS)
Aantal: 1









schaal 4:1		datum 30-6-2016	opmerkingen
maateenheid mm			
getekend Ilona Brouwer			
groep GIP		gewicht 107 gram	
benaming drawning_construction3__block_control			
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