Project Number: 257401

A highly integrated and sensitive POrous SIlicon based lab on a chip for multiple quantitaTIVE monitoring of food allergies at point of care.

Specific Targeted Research Project

Information Society Technologies

Deliverable D5.1: Report on the design for optimised serum preparation and injection system

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

1 About this deliverable

1.1 Introduction

In POSITIVE a POC system will be developed which can take whole blood samples form a patient to measure the level of allergic reaction to a set of food allergens. This report deals with a) the transfer of blood sample from the patient into the POC system (sample injection) and b) the transformation of the whole blood into serum or plasma (optimized serum preparation).

1.2 Scope of the deliverable

A blood sample will be transferred from the patient finger into the Positive device sample input. From there, the blood goes through a filter which retains the hematocrit. The resulting serum is then led into the porous membrane where the immunoassay and detection take place. This report focuses on the design of the device input and filter. However, all fluidic operations in the device are tightly coupled, and the design will therefore consider the whole process from patient finger to successful sensor read-out.

The process steps for the usage of the POC system will be the following

1. Transfer of blood sample from patent (finger) to cartridge inlet
2. Filtering of blood serum or plasma
3. Mixing of serum with buffer (and reagents?)
4. Transport of serum/buffer through porous sensor membrane
5. Rinse to reduce non-specific binding
6. Preparations for sensor read-out (if any, mechanical, etc).

1.3 Structure of this deliverable

The report is laid out according to the following topics:

2 Transfer of blood sample from patient to device inlet

A product like One-touch Lancet Device (1) can be used for skin perforation (see Figure 1). These devices carefully puncture the finger skin to achieve the needed amount of blood, using a disposable lancet.

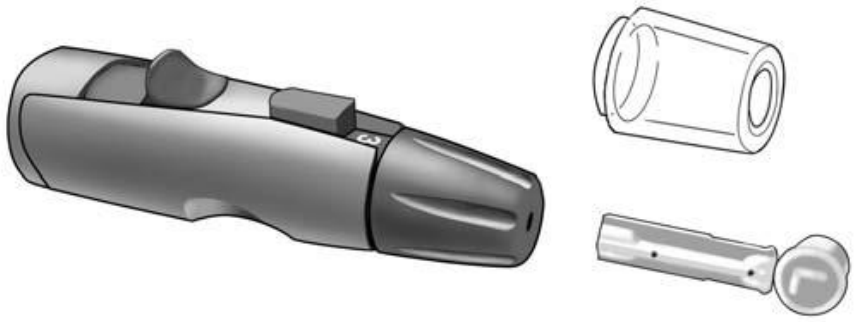

Figure 1: One-touch lancet device from LifeScan Inc.

A simple, sterile pipette can be used to transfer a blood sample from the patient's finger after skin perforation. For instance, Bailey offers a 0.3 mL Pedriatic Pipet (2) in their Disposable Transfer Pipets portfolio (see Figure 2).

Figure 2: Disposable transfer pipette from Bailey

As long as the transferred volume of blood is not considerably reduced by the device, the selection of transfer device can be done independently of the cartridge design. It must be kept in mind that the targeted amount of blood from the patient is 100 uL, and loss during transfer should be minimized to allow a sufficient and precise amount of blood sample to be introduced into the Positive device. One requirement is that the cartridge inlet opening is large enough to accommodate the device tip or allow a dispensed blood droplet to be sucked into the cartridge. A large cartridge inlet opening diameter of 6 mm will be used in the design, so this will in practice not pose any restrictions.

3 Filtering of blood serum

3.1 Requirements of the filter solution

The immunoassay at the core of the allergy detection is not compatible with whole blood. Red and white blood cells and platelets must be removed from the sample, which then will consist of the remaining blood plasma. Depending on the existence of coagulation factors and timing, the filtered sample will be equivalent to the blood serum traditionally obtained by centrifugation.

It is assumed that the addition of anti-coagulants is not necessary. Blood might coagulate before or during contact with the cartridge filter. The filter will trap blood cells and fibrinogens (activated fibrines and coagulation factors), and let what is equivalent to blood serum through. On the other hand, if blood does not coagulate in this time, the filter will only retain the blood cells and let remaining plasma (including clotting agents) through.

3.2 Whole-blood filtering in the literature

Scientific publications on filtering of whole blood range from those reporting novel methods of filtering, to those describing the integration of traditional filters into complete sensing systems. Some of the most interesting work will be reviewed here together with an evaluation of their possible application in the Positive project.

Bonanno (3) describes the use of porous silicon as both filtering and detection device. The pore diameters were in the order of 100 nm (see Figure 3). The blood sample was in this case mixed with anti-coagulant and applied to the PSi in a humidified enclosure. After rinsing, reflection spectroscopy was used to detect the wavelength shift caused by the pore filling.

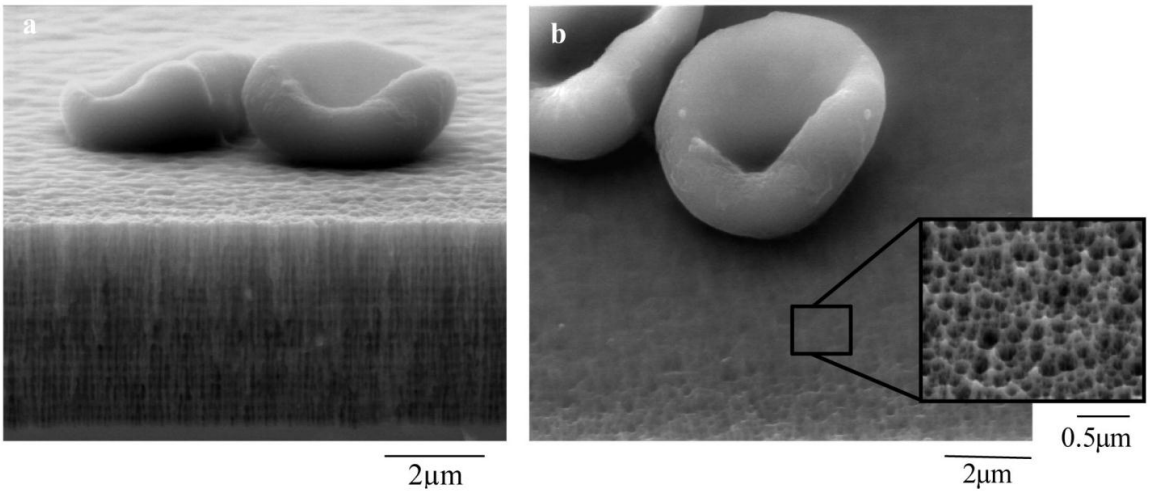

Figure 3. Red blood cells resting on porous silicon membrane, from Bonanno (3).

This approach can be an alternative for the Positive device since we already have a similar porous silicon membrane. However, the process times involved in sample incubation and rinsing (1 hour in the referenced work) might be too long for our application.

Shim (4) shows a hetero-packed bead filter, where the small beads filter the blood and the large beads prevent the small beads from entering the outlet channel (see Figure 4). Capillary forces drive the plasma through the 100x100 µm outlet channel at a speed of 0.25 uL/min.

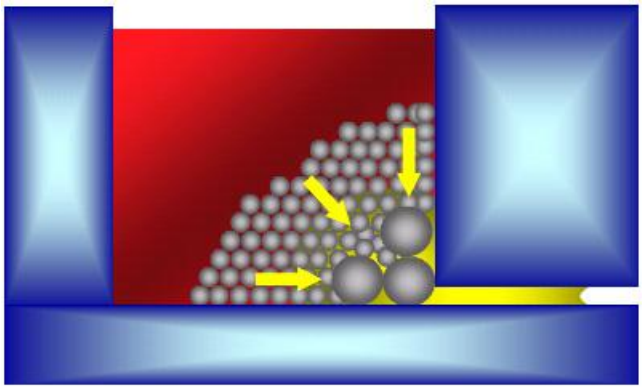

Figure 4. Hetero-packed bead filter, from Shim (4).

The preparation of the filter, including the positioning and fixation of the bead packs, brings some complexity and possible risks. The use of a vaccum pump to suck the beads into place is viable in a production facility, but if the beads are deattached and misplaced during storage, the filter will leak in subsequent usage.

Crowley (5) uses silicon and glass MEMS structures in form of a planar microfilter of 0.5 um high pores (see Figure 5). The need for complex MEMS fabrication makes this approach

less attractive in our design. It should however be considered whether such filter structures can be integrated into the sensor array fabrication in work package 7.

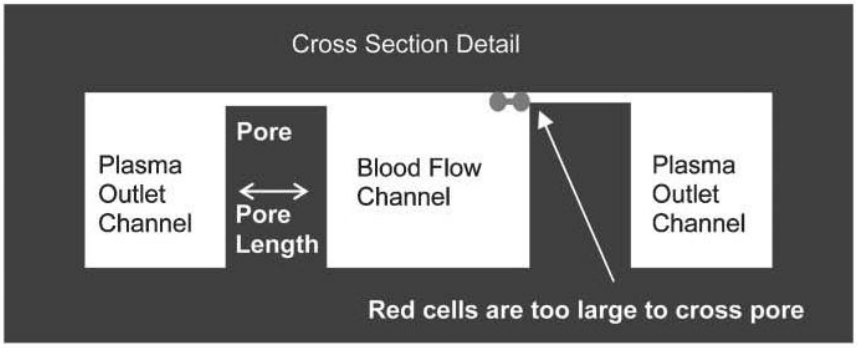

Figure 5. MEMS-fabricated microfilter, cross-section, from Crowley (5).

Weigl (6) reports how Micronics Inc. uses diffusion-based separation on a hematology cartridge device (see Figure 6). Using a hydrostatic-pressure or capillary effect driven laminar flow, different compounds are separated by their diffusion coefficients. However, plasma separation is not demonstrated, and the filtering effiency is incertain.

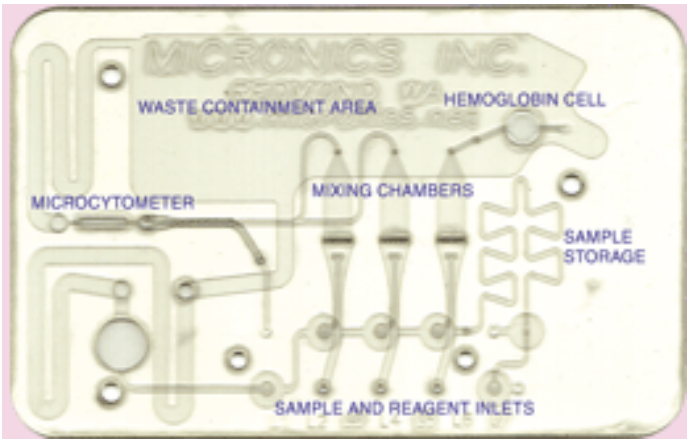

Figure 6. Hematology Cartridge from Micronics (6).

Thorslund (7) integrated several different filtration membranes into a on-chip system (see Figure 7). All filters were tested with syringes to drive the liquid. Best results were achieved with a PVP/PES filter from PrimeCare which however is not available any longer.

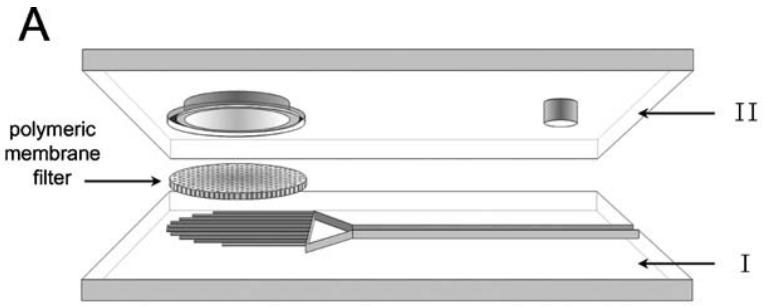

Figure 7. On-chip blood filtering system from Thorslund (7).

As in most reports on flow-through filters, the membrane filters were tested with external pumps (vaccum, syringe) without reference to the applied pressure. So little information could be collected on the needed pressure and pressure drop across the filter membranes.

Another proven method of filtering whole blood is centrifugation; however, this would need rotating parts and loading mechanisms and add significant mechanical complexity to the device.

Of the surveyed approaches, the use of commercially available filter membranes seems to be the simplest and most promising way of designing a disposable, built-in filter. Although the referenced PVP/PES membranes can not be found today, also other commercial membranes have features such as filter pore gradients to reduce clogging effects.

3.3 Commercially available membranes for whole blood filtering

Membrane filters allow a simple design and since several filters for whole blood separation exists on the market, this approach has been followed. Products from several manufacturers have been evaluated for their suitability.

Pall Corporation produces a number of filtration membranes for blood filtering found under their Life Sciences / Diagnostics section. The Pall CytoSep Membrane (8) is designed for separating plasma from whole-blood, although its description states that also platelets and leukocytes move through the membrane.

The Pall Vivid Plasma Separation Membrane (9) has an asymmetric pore structure to efficiently filter cells without lysis. Further, it is described as having low protein retention, which will be important for a subsequent quantitative biomarker [be specific] assay like in the Positive device.

The Pall Vivid 170 Nitrocellulose Membrane (10) is tailored for lateral flow assays. It has a polyester backing which would make it unusable as a flow-through filter. However, if lateral filtering can be considered, this membrane is a promising candidate.

Whatman Ltd. offers under their Diagnostic Components section (11) membranes for flow-through (vertical) blood separation. In particular the MF1 product seems suitable for our sample volumes, less than 100 uL.

Millipore does not indicate any product especially targeted for whole-blood filtration. Their Durapore PVDF membrane (12) is a low protein-binding membrane and is available in different pore sizes.

On the basis of their specifications, the Pall Vivid, Whatman MF1 and Millipore Durapore membranes will be tested as part of the serum preparation filter in the Positive device. For the Pall Vivid, two different grades will be evaluated, GF and GX.

4 Design of injection system

An optimal design of the injection system would not consider an isolated injection module to be later coupled with the rest of the device. A realistic and efficient design encompasses the total work flow and tries to reduce the number of components needed. As such, the presented design for the complete device minimizes the number of liquid transport channels and interfaces and addresses the whole process from patient sample insertion to successful sensor readout.

On the other hand, for the preliminary evaluation of the filter solution an intermediate design will be presented where individual performance of the components can be more easily characterized.

4.1 Componentized approach

A basic and flexible approach consists in linking individual components (injection and filter unit, sensor membrane, buffer reservoir, waste reservoir, pumps or other driving units) together with fluidic channels. The channels can be hydrophilic and structured to optimize capillary flow, or depend on a pump unit to push or pull liquids through the components. The flow circuit must be sealed, in particular around the sensor membrane, where also the optical read-out is performed. The material of the channel walls must therefore not interfere with the read-out beam. At this point, the useful signal wavelengths and consequently, the needed transparency properties, have not been determined.

Depending on the flow resistance of the resulting liquid circuit, pressure differences may dictate a strong sealing around components. In particular, tight sealing of the sensor membrane can be complicated by its fragility, for instance, preventing a directly clamping sandwich structure.

4.2 Direct sample/buffer injection system

In order to avoid some of the issues raised in the componentized approach description, a more compact and simple approach will be presented.

Advantages:

- Short/zero flow path between filter and sensor membrane
- Saves time and sample volume
- Few/no optical obstacles during sensor read-out
- No destructive sealing forces on sensor membrane perimeter
- Reduced material complexity and cost

The process as executed by the user will consist of the following steps:

1. Remove protective foil
2. Inject blood sample into opening in filter lid (directly on filter membrane)
3. Wait X seconds for serum to diffuse into sensor membrane
4. Remove filter lid
5. Dispose buffer solution onto membrane (pre-dosed in a disposable pipet, or a pipet with precise dosage)
6. Insert cartridge into read-out device
7. Wait while device performs buffer flushing and readout
8. Receive results on display or connected printer/computer

The read-out device powers up and activates on cartridge insertion. During the flushing step, a liquid/humidity sensor beneath membrane can detect buffer traversal and dry-out of membrane, if a time-based algorithm does not show to be sufficient.

Since all specifications of the optical read-out have not been established at this point, several alternative designs are presented. In particular, the choice between reflective or transmissive read-out will be important for the final design of the sample processing.

4.2.1 Case 1: Reflective optical read-out

The serum sample will fill the membrane by the capillary action of the membrane itself. In the subsequent buffer flush, an absorbing pad directly underneath the membrane will pull the buffer through the membrane. The pad is part of the disposable cartridge, and must be large enough to quickly absorb the buffer volume.

To ensure good wicking properties from the membrane to the pad, they must be in direct contact. If the fragility of the membrane is incompatible with the pad material or contact geometry, an intermediate layer of softer wicking yarn can be used.

4.2.2 Case 2: Transmissive optical read-out

In this case an optical transmitter or receiver will have to be placed below the sensor membrane. An absorbing pad would be an optical obstacle. There are several possibilities:

1. Removing the pad before inserting cartridge into read-out device
2. Removal of pad inside read-out device
3. Transparent channel between membrane and pad

Solution (1) would be the simplest from the device point of view. It will require two additional steps in the original user process list:

5b) Wait for buffer to flow through

5c) Remove pad

Solution (2) can be achieved by a mechanism tearing off the pad. It could also be constructed like a “drawer” which the user pulls out after the required waiting time.

In solution (3) the liquid must be transported from the membrane to the pad by means of either capillary forces or a driving pump. To obtain capillary transport while preserving optical transparency, the walls should be flat and be closely separated.

4.3 Description of design

An exploded view of the cartridge assembly can be seen in Figure 8. A protective sheet of thin plastic film, to be removed before injection of the blood sample, is not shown.

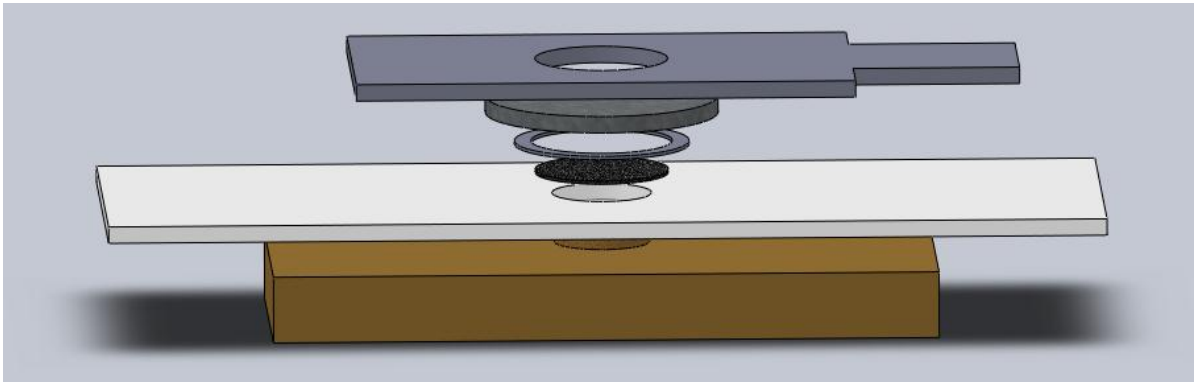

Figure 8. Upper parts of cartridge, exploded view. The parts are from top to bottom: a) Filter lid with rip-off handle tag, b) Filter membrane, c) Protection ring, d) Sensor membrane, e) Cartridge base, f) Wicking layer, g) Absorbing pad

The user injects the blood sample into the opening in the filter lid, and after the filtrated blood serum has immersed the membrane filter, tears of the filter lid to leave the sensor membrane exposed.

4.4 Risks

Some of the processes involved are not yet fully determined and others are, at this early point in the project, not well characterized. Consequently, there is the risk that the above direct sample/buffer approach will not be sufficient. In this case elements from the componentized approach can be gradually introduced, with greater flexibility and individually proven functionality. On the other hand, the cost of greater system complexity, and the individual risks of the added elements justify the pursuit of the simplest solution. Risks factors can be identified already, with suggested fallback solutions or remedies.

Capillary forces in the sensor membrane may not be sufficient to fill the membrane pores within adequate time frames. This can be a result of unfavourable (hydrophobic) surface properties of the polymer coating on the pore walls, and the final pore size.

- This will require the application of a driving pressure. An overpressure from the top can be provided through a chamber residing in the read-out device sealing down on the cartridge. A mechanism can flip this chamber away before the optical read-out is performed. Alternatively an underpressure can be applied below the cartridge, which in the case of a reflective read-out will suppress the need for a flip mechanism. The maximum relative pressure will be limited to atmospheric pressure and reduced further by the efficiency of the pump and the tightness of the chamber.
- The cartridge design can remain unmodified
- The user will have to insert the cartridge twice, removing the filter lid in between

4.5 Liquid channel for fallback solutions

If transmissive optical read-out proves to be necessary and, at the same time, removal of the absorbing pad is not practical, the pad must be positioned away from the optical axis. A

transparent flow channel can be incorporated below the sensing membrane to lead the liquid to the pad.

In the case an underpressure needs to be applied in order to help driving the liquids through the sensor membrane, a sealed chamber must be formed below it. The same flow channel can be the basis of this chamber. The absorbing pad is not necessary, but a similar pad can be used as waste reservoir, to be pulled out and disposed of after the finished measurement.

The liquid channel is formed by a channel engraved in the cartridge holder itself, sealed off by a transparent cover tape (see Figure 9).

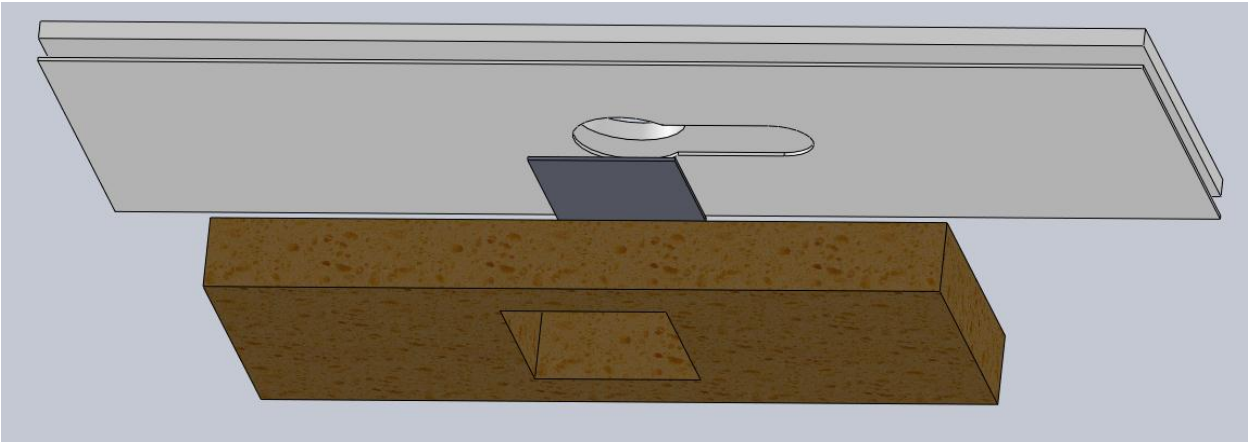

Figure 9. Liquid channel at the lower side of the cartridge, exploded view. From top: a) Cartridge base, b) Channel gasket, c) Tape window, d) Absorbing pad. The channel gasket can alternatively be replaced by a shallow channel engraved directly in the cartridge base.

5 Preliminary testing of filters

A fluidic device has been designed to allow evaluation of different membrane filters under conditions close to those of the complete system.

A testing rig for the sensor membrane has already been fabricated, which will allow sealed flow-through experiments (see Figure 10). In addition to characterization of flow dynamics of the membrane, also functionalization tests can be performed with this device.

Figure 10. Test rig for sensor membrane flow experiments. The two tubes on the left and right hand side is the inlet and outlet respectively. The sensor membrane is fixated with sealing tape on a holder which again is clamped between the transparent top and bottom cover plates. Also visible on the drawing is the four lugs pointing towards the centre which provide mechanical support for the membrane.

The same device can be used for filter testing, by replacing the sensor membrane with the filter membrane. The transparent cover plates allows for visual inspection of the filter surface during the experiments.

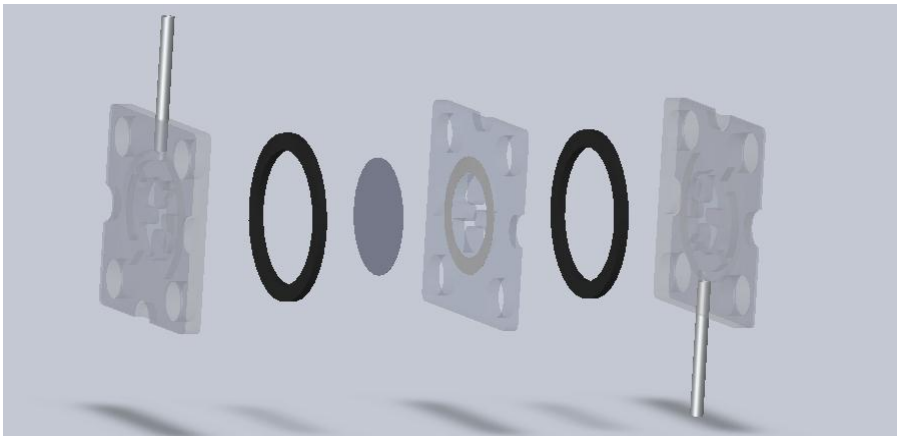

Figure 11. Filter membrane testing device, exploded view.

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