Fourth Year Honours Project
for the Degree of
M.Eng. in Mechanical Engineering and Energy Engineering

Journal Paper

**Nithiya Mangala Streethran**

H00158233

# Fabrication of paper-based microfluidic devices for animal disease detection

29th April 2016

Project Supervisor: Dr. Will Wenmiao Shu

**Heriot-Watt University**
**School of Engineering and Physical Sciences**
**Mechanical Engineering**

# Fabrication of paper-based microfluidic devices for animal disease detection

**Nithiya Mangala Streethran**

H00158233

M.Eng. in Mechanical Engineering and Energy Engineering

School of Engineering and Physical Sciences
Heriot-Watt University
Edinburgh
EH14 4AS
United Kingdom

Project Supervisor: Dr. Will Wenmiao Shu

29th April 2016

Email: nms31@hw.ac.uk

---

***Abstract*–** The main features of paper-based microfluidic devices are hydrophilic channels surrounded by a hydrophobic agent which leads to test zones containing reagents for colorimetric tests. These devices are used in diagnostics as they are small, lightweight, inexpensive, rapid and are point-of-care devices which only require a small volume of fluid. Paper has many advantages, including having the ability to transport liquids through capillary action. For animal disease diagnostics, a device which is able to test a large number of samples quickly and provides accurate results is necessary in order to identify and prevent further spread of diseases. The aim of this project is to develop paper-based microfluidic devices to be used extensively in biomedical applications for animal disease diagnostics while also addressing the key challenge faced by researchers with regards to three-dimensional devices in transporting fluid vertically through multiple layers. This journal details activities including literature review, design, fabrication, testing and results, completed throughout the course of the project as well as further activities and recommendations for the future. A number of prototypes were made for the three design concepts and testing produced promising results solving the key challenge.

**Keywords**: microfluidics, paper, diagnostics, animal diseases, laser cutting, point-of-care testing, low-cost, biomedical applications, lab-on-a-chip

# Table of Contents

## Table of Figures

**Figures**

**Tables**



**Equations**



## Nomenclature

| Symbol | Meaning | Unit |
|---|---|---|
| T | Thickness of material | mm |
| t | Approximate time taken for fluid flow | min and sec |

# 1 Introduction

The main characteristics of conventional microfluidic technologies are that only a small volume of fluid sample is required (in the order of microliters), the devices are small and the testing is point-of-care, which means the testing is done and results are obtained right beside the patient. [1, 2] These characteristics make these technologies ideal for when fluid samples are limited, refrigeration facilities are unavailable and the sample cannot be transported. [3, 4]

Paper-based microfluidic devices are being used to diagnose diseases in humans in developing and underdeveloped countries as they fall under the World Health Organisation's ASSURED criteria – affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and delivered. [5, 6] The same criteria would apply for animal disease testing [7, 8], especially livestock, as a large number of tests is expected to be conducted on a large number of animals with limited sample volumes to identify and control the spread of an infection in the event of a disease outbreak [9]. Veterinary diagnosis is important in controlling the spread of zoonotic diseases to humans which can lead to infections [10, 11] such as influenza and HIV, and cause pandemics. As animals are a source of food, veterinary diagnosis would also control foodborne diseases resulting from the consumption of dairy, eggs and meat infected with bacteria and viruses [3, 12].

Paper has many advantages. It is abundant and inexpensive and it allows for safe disposal of used devices through incineration, which is crucial since biohazards, and chemicals are involved. [13] Paper is a porous medium and fluid flows only through capillary action without the involvement of external forces due to the cohesive and adhesive properties of paper. [5, 14]

Modern day paper-based microfluidics is associated with the Whitesides Research Group of Harvard University in 2007 [13]. The group patterned paper using a hydrophobic patterning agent to create hydrophilic channels that guide a fluid sample to flow to test zones independent of each other. [1, 15] Test zones contain reagents which cause a change or appearance in colour if a specific substance is present. Three-dimensional paper-based microfluidic devices consist of multiple layers and the key challenge in fabricating them is allowing fluid flow vertically from one layer to another by providing contact points between layers. [3]

This project focusses on developing paper-based microfluidic devices to be used extensively in biomedical applications for animal disease diagnostics while also addressing the key challenge with regards to three-dimensional devices in transporting fluid vertically through multiple layers.

# 2 Literature Review

## 2.1 The Use of Paper

Paper has many advantages as explained briefly earlier. Chromatography and filter papers are widely used in paper-based microfluidics [16, 5, 17, 15] as cotton of high quality with over 98% α-cellulose content is used to make these papers, making them biologically compatible and pure [18, 3]. As paper is white in colour, it is able to provide a strong contrast for colorimetric tests. [19] Pore size of paper is an important property which affects fluid flow as large pores may cause hydrophobic agents as well as fluids to diffuse uncontrollably, resulting in the swelling of cellulose fibres and subsequently constriction of capillary flow. [3, 20] The surface area of paper is directly proportional to its thickness provided other parameters are constant. [21, 22]

## 2.2 Existing Devices

### 2.2.1 Two-Dimensional Devices

Two-dimensional devices are single-layered and rely on lateral fluid flow. **Figure 1** shows a simple two-dimensional device which tests the presence of glucose and protein in urine [23]. The figure also shoes the main features of the device: hydrophilic paper channels which are formed by hydrophobic barriers surrounding them and guide to test zones that contain reagents.

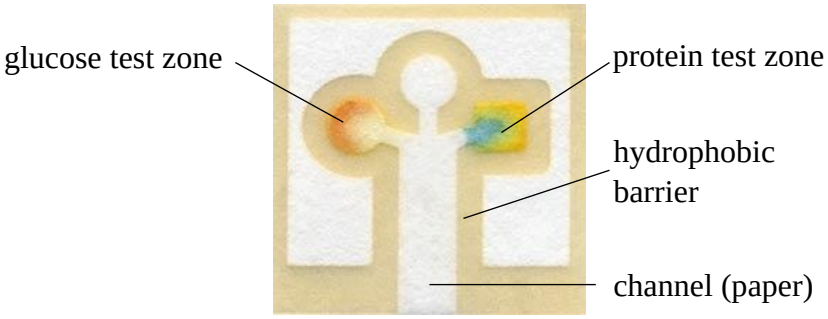



***Figure 1**: Labelled image of a two-dimensional paper-based microfluidic device showing its main features*
*Adapted from [23]*

The patterned shapes vary from simple geometric shapes to more complex shapes as shown in **Figure 2**.

(a) (b)

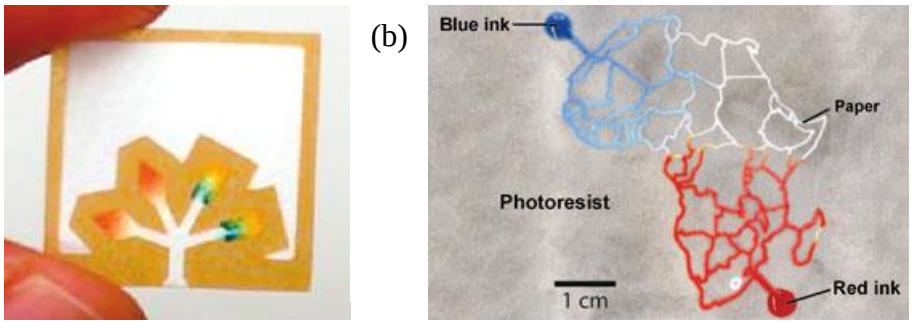



***Figure 2**: Two-dimensional paper-based microfluidic devices*
*patterned using photoresist (a) testing glucose and protein; (b) in the shape of the African continent*
*Adapted from [5] and [24] respectively*

**Figure 3** shows a two-dimensional paper microzone plate, a device based on the standard 96-well microtiter plate shown in **Figure 4**. These plates are made of polymers, approximately

11mm thick [25] and widely used in biomedical applications, especially in enzyme linked immunosorbent assays (ELISA), making use of equipment such as multichannel pipettes (**Figure 5**). Although these plates are recyclable, a safe disposal method and low cost alternatives are needed in laboratories where resources are limited. [19, 5, 13] Plates made of paper allow for thinner, low-cost devices that are easily manufactured and disposed and require reduced fluid volumes in each well (approximately 10µl compared to 70µl for polymer plates). [19, 5] The test fluid will quickly evaporate causing it to concentrate as these test zones are exposed to the air and have high surface area to volume ratio. [19, 5]

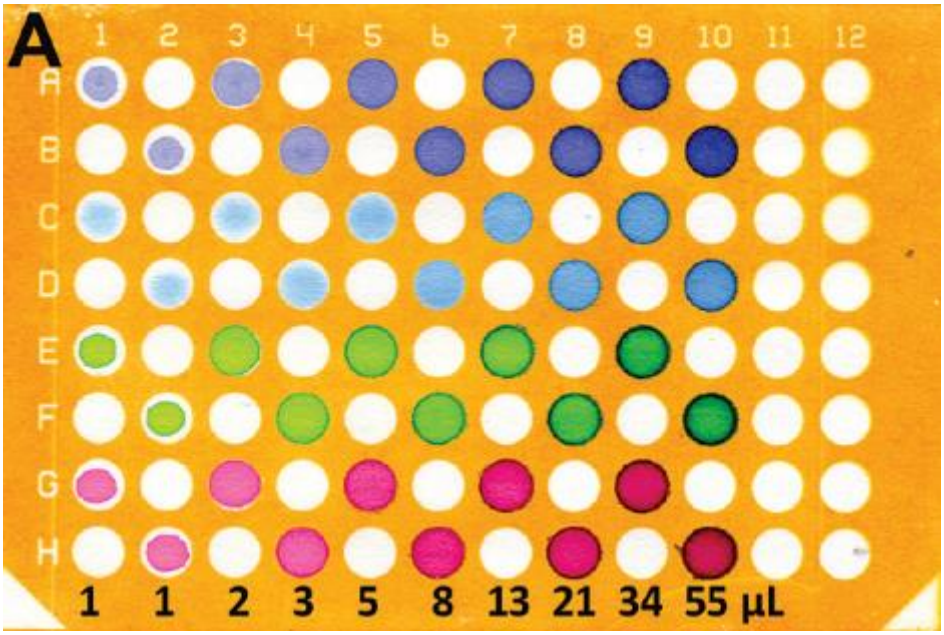



***Figure 3**: Paper microzone plate with 96 zones*
*Adapted from [19]*

***Figure 4**: Standard 96-well microtiter plate*
*Adapted from [26]*

***Figure 5**: Eight-channel multipipette*
*Adapted from [27]*

### 2.2.2 Three-Dimensional Devices

Three-dimensional paper-based microfluidic devices are multi-layered and allow for more complex analyses to be carried out. These devices consist of multiple patterned paper layers similar to that of a two-dimensional device but stacked with alternating layers of adhesives with holes at the hydrophilic areas that provide contact between layers. Fluid flows vertically from the inlet on the top layer to the bottom layer containing test zones. **Figure 6** shows three examples of existing three-dimensional devices. Each inlet and fluid path is indicated by a different colour. In device (c), blue dye solutions of different concentrations were added to each inlet, showing different intensities of colour.

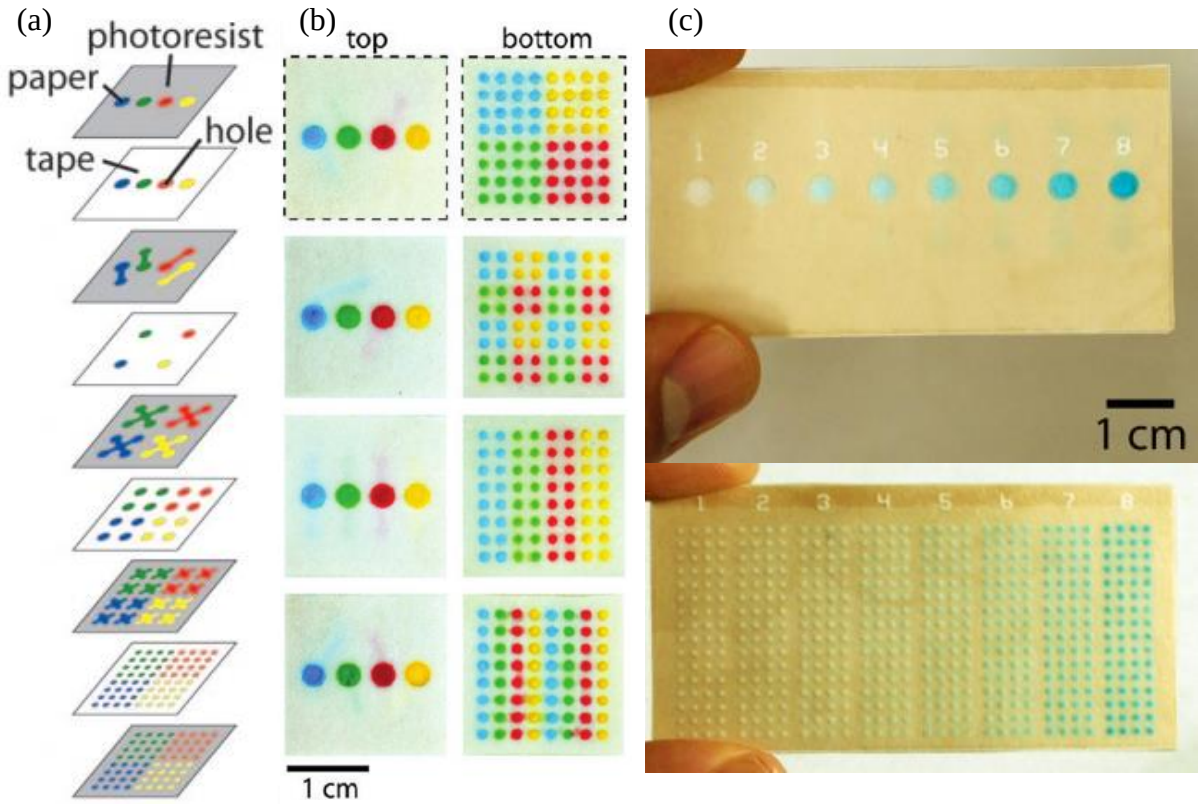



***Figure 6**: Three-dimensional paper-based microfluidic devices*
*(a) exploded view of a CAD model of a device with five layers of paper patterned with photoresist; (b) the top and bottom layers of four devices with four inlets each that form sixty-four test zones in total; (c) top and bottom views of a device with eight inlets and sixty-four test zones each and tested using an eight-channel micropipette with different concentrations of erioglaucine*
*All figures adapted from [28]*

As mentioned, three-dimensional devices rely on vertical fluid flow from the inlet to test zones. To achieve this, contact points between hydrophilic features of adjacent layers are necessary which is often prevented by gaps in between due to the features being thinner than the hydrophobic areas after adhesive is applied and the layers are assembled. As paper is made primarily of cellulose, cellulose powder or cut paper are good choices to fill the spaces, shown in **Figure 7**. [3, 5, 13] However, this has to be done manually and can be difficult for devices with small hydrophilic areas. Another method would be to laminate the assembled device. Hot lamination after the addition of reagents may cause the reagents to denature, especially if there are enzymes and a range of working temperatures. Furthermore, lamination produces stronger devices and prevent contamination or evaporation of reagents in the test zones. Other methods include the use of clamps and the addition of an absorbent layer which absorbs all excess fluid and prevent leaks. [3]

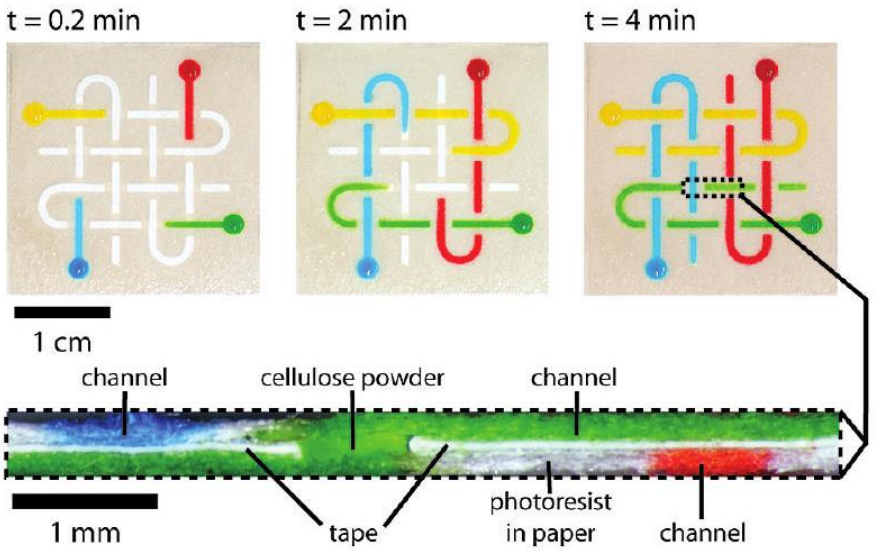



***Figure 7**: A three-dimensional device and its cross-section showing the use of cellulose powder in the holes of the tape to allow vertical flow of fluid*
*Adapted from [5]*

## 2.3 Fabrication Methods

Paper-based microfluidic devices can be fabricated through two general approaches: selective hydrophobisation, and selective dehydrophobisation. [13] The former is done by depositing a hydrophobic agent on certain areas of hydrophilic paper to form boundaries. Examples of hydrophobic agents are photoresist, wax, polystyrene and alkyl ketene dimer and the types of paper used include chromatography paper and filter paper. The latter is done by removing part of hydrophobic agent on hydrophobised paper to form hydrophilic channels. The types of paper used include parchment paper and wax paper, where the hydrophobic agents are silicone and wax respectively. [3, 5, 13] These methods fall under the principle of blocking pores in paper physically. Another principle would be cutting paper in shape using a laser cutter, [13] where lamination or other backings must be done to provide rigidity to the device. [3]

## 2.4 Testing and Analysis

Qualitative testing checks if a particular substance is present and results in either a positive or negative outcome. An example is the testing of glucose in blood or urine using the glucose oxidase-peroxide reaction, which will form a pink dye if glucose is present. [29]

Dye solutions are used in testing the devices as the solutions come in a variety of colours with known concentrations. Examples of dye used are tartrazine (yellow and green) erythrosine and erioglaucine (blue). [17] Devices tested using known concentrations of a dye can be analysed using imaging software such as ImageJ and Adobe Illustrator. Imaging involves determining the mean intensity of colour of test zones, calculating the average intensity and subtracting the intensity for an assay with a concentration of zero from the average. These are then plotted against the concentration to form calibration curves, shown in **Figure 8**. When an assay of unknown concentration is tested, imaging can be done in the same way and the unknown concentration is found using the intersection point between the intensity and the calibration curve. Besides qualitatively testing the flow of a fluid sample laterally or vertically in the case of three-dimensional devices, the use of dye solutions of known concentrations would provide quantitative information on flow rate and distribution of the solution, showing its effective concentration at the test zones needed to evaluate the performance of the assay. [3, 22]

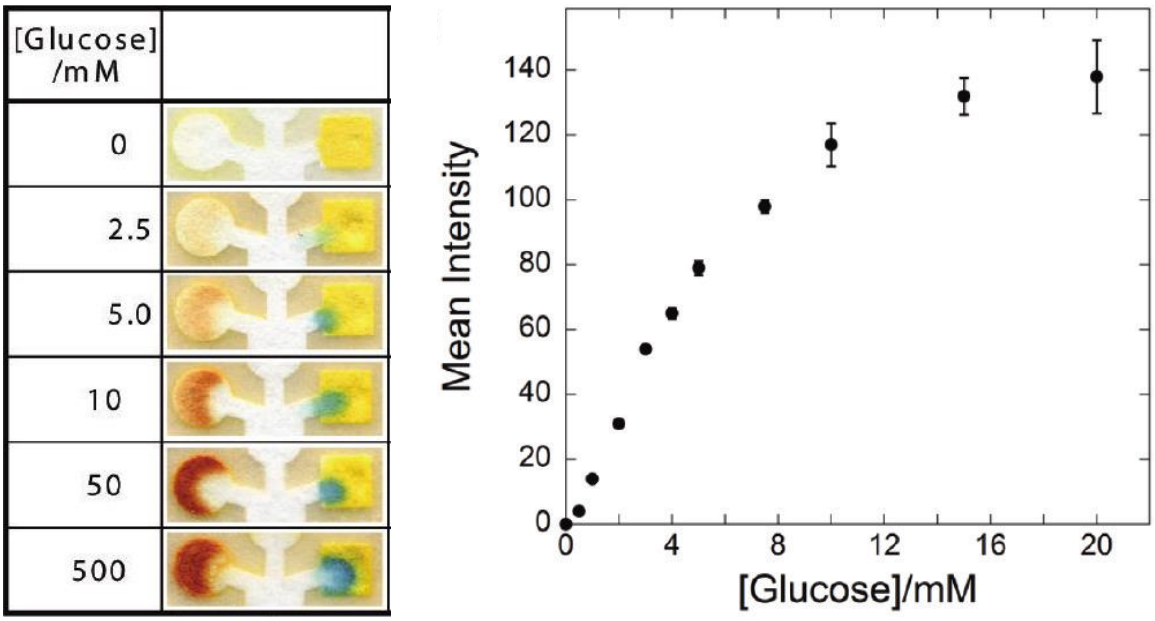



***Figure 8**: Results obtained through testing paper-based microfluidic devices with a range of concentration of glucose in urine and the corresponding intensity versus concentration calibration curve*
*Adapted from [15]*

### 2.4.1 Animal Disease

Types of livestock include pigs, cattle, poultry and birds, and small ruminants such as goats and sheep. Fluid samples that are commonly used to test diseases are blood, serum, urine, saliva, foetal fluid, mucus, milk and faeces. These samples can be tested for bacteria, virus, parasites or other pathogens, pregnancy, infectious diseases, tumours, glucose, protein and pH levels. [30, 31, 3] Semi-quantitative testing may be done to evaluate the pH of urine. Typically, the urine of ruminants is alkaline but may vary if the animal has abnormalities or is under medication. [32, 33, 34]

Rapid testing of diseases is done for quick evaluation of animals in small numbers and would normally take 10 minutes to 2 hours. Rapid results are important to immediately prevent further spread of diseases. For more specific and sensitive devices, ELISA is used which requires further analysis [35] such as imaging explained earlier. An example of a contagious disease that affects ruminants and pigs is the foot-and-mouth disease. Antibody-detecting ELISA is

used to qualitatively analyse the presence of an antibody called anti-3ABC in blood and serum of the animals to differentiate infected from vaccinated animals (DIVA). A negative result indicates the animal is healthy or vaccinated, while a positive result indicates an infected animal. [36]

# 3 Chosen Fabrication Method

Based on the availability of equipment and training at university, wax printing and laser cutting followed by lamination were the two fabrication methods considered.

Wax printing is an inexpensive [16] fabrication method involving the use of a printer modified to print wax patterns onto paper. [13] To form the hydrophobic barrier, the printed paper is then heated in an oven which causes the wax to melt and diffuse through the thickness of the paper, as shown in **Figure 9** below. It can be seen however that the barrier is wider on the top where the wax was deposited during printing, then on the bottom due to lateral spreading of wax in paper which occurs more rapidly than vertical spreading. [16, 3]

The other fabrication method is paper cutting followed by lamination. As shown in **Figure 10**, paper is cut in shape of the hydrophilic channel and holes are cut in lamination pouch which consists of two sheets of plastic fused together. These are done using a laser cutter and the holes are slightly smaller than the paper. The paper is then aligned inside the pouch and laminated. Since the paper is cut in shape, the boundary between the hydrophilic and hydrophobic areas are very clear since the lamination pouch is hydrophobic and no patterning agents were used. The use of these two materials also causes the thicknesses of the hydrophilic and hydrophobic areas to differ, allowing for more methods to reduce gaps between layers and increase contact in three-dimensional devices to be experimented with. The test zones on both sides are exposed to air, enabling reagents to be added after lamination.

Due to this and as training was readily available, the paper cutting and lamination method was chosen. The materials that will be used are Whatman Grade 1 Chromatography Paper (see Appendix B.i: Chromatography Paper for data sheet) and Staples Lamination Pouch (see Appendix B.ii: Lamination Pouch for data sheet). This grade of chromatography paper is widely used [5] and has medium porosity, thickness of 0.18mm and pore size of 11μm, [37] giving the paper enough tensile strength and preventing paper swelling or breaking.

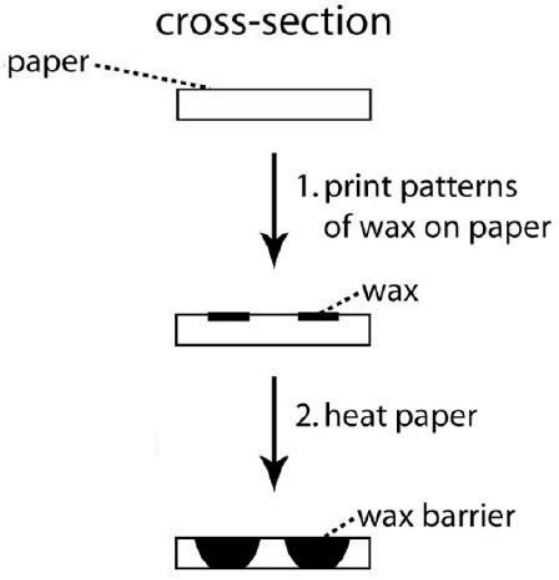



***Figure 9**: Wax printing method of fabricating paper-based microfluidic devices*
*Adapted from [5]*

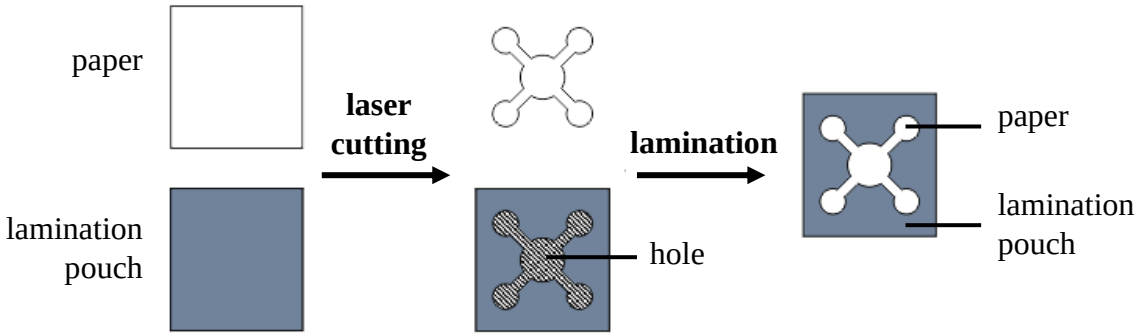



***Figure 10**: Laser cutting and lamination method of fabricating paper-based microfluidic devices*

# 4 Design

## 4.1 Design 1 – Three-Dimensional Multi-Zone

The first concept is a three dimensional device consisting of four layers as shown in **Figure 11**. The device measures 50mm × 50mm and has four inlets on the first layer, each indicating a different colour. Each inlet is able to test different samples. Alternating layers of adhesive with holes secure the layers in place. The fourth layer has eighty-four test zones in total, again shown in different colours, indicating twenty-one tests can be done from each sample. These test zones are independent of each other, which means each zone may have a different reagent testing different substances in the same sample, reducing the amount of materials, fluid volume and time required to conduct each test. The test zones may also contain the same reagents testing the same substances, producing duplicate results which will potentially reduce possible errors in qualitative testing. As the total area of paper leading from each inlet is equal, the same volume of liquid can be used for each inlet, making it standard and suitable for quantitative tests and for biomedical applications in general. This design aims to solve the aforementioned problem involving fluid flow in three-dimensional devices. See Appendix A.i: Design 1 for engineering drawings of the design.

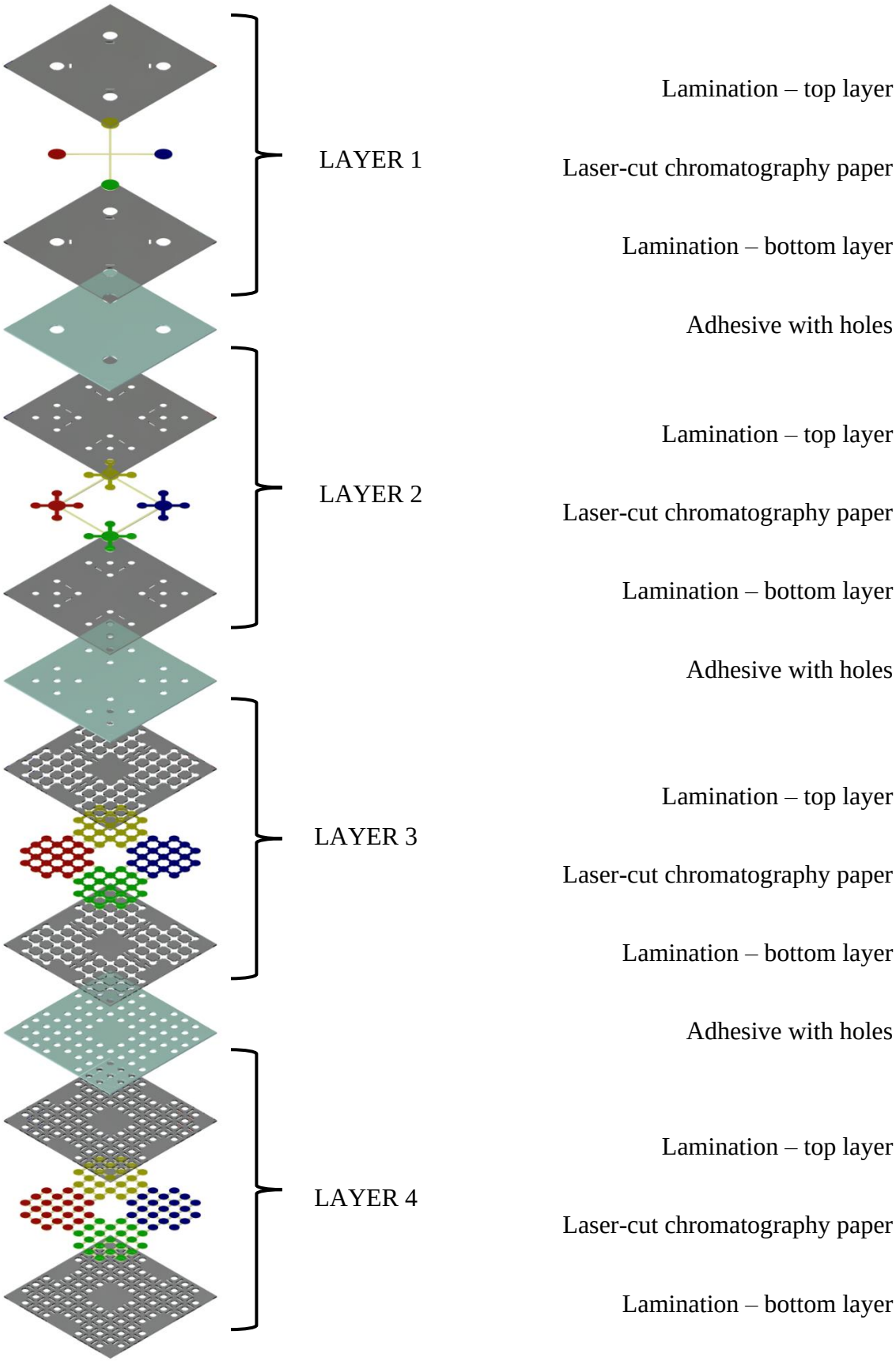



***Figure 11**: Design 1 – Three-dimensional multi-zone*

### 4.2 Design 2 – Heriot-Watt University's Shield-of-Arms

To test the complexity of designs that can be fabricated, a design featuring Heriot-Watt University's shield-of-arms was created (see **Figure 12**). It measures 50mm × 50mm and consists of three layers with two inlets and multiple test zones which are irregular in shape. It is simplified into including the tree, cinquefoils, book and star only, as sketching the design is difficult due to the irregular shapes of the components, as opposed to Design 1 which only consists of circular shapes. The design was sketched on Creo Parametric 3.0 by superimposing an image of the university's shield and tracing the outline. See Appendix A.ii: Design for engineering drawings of the design.

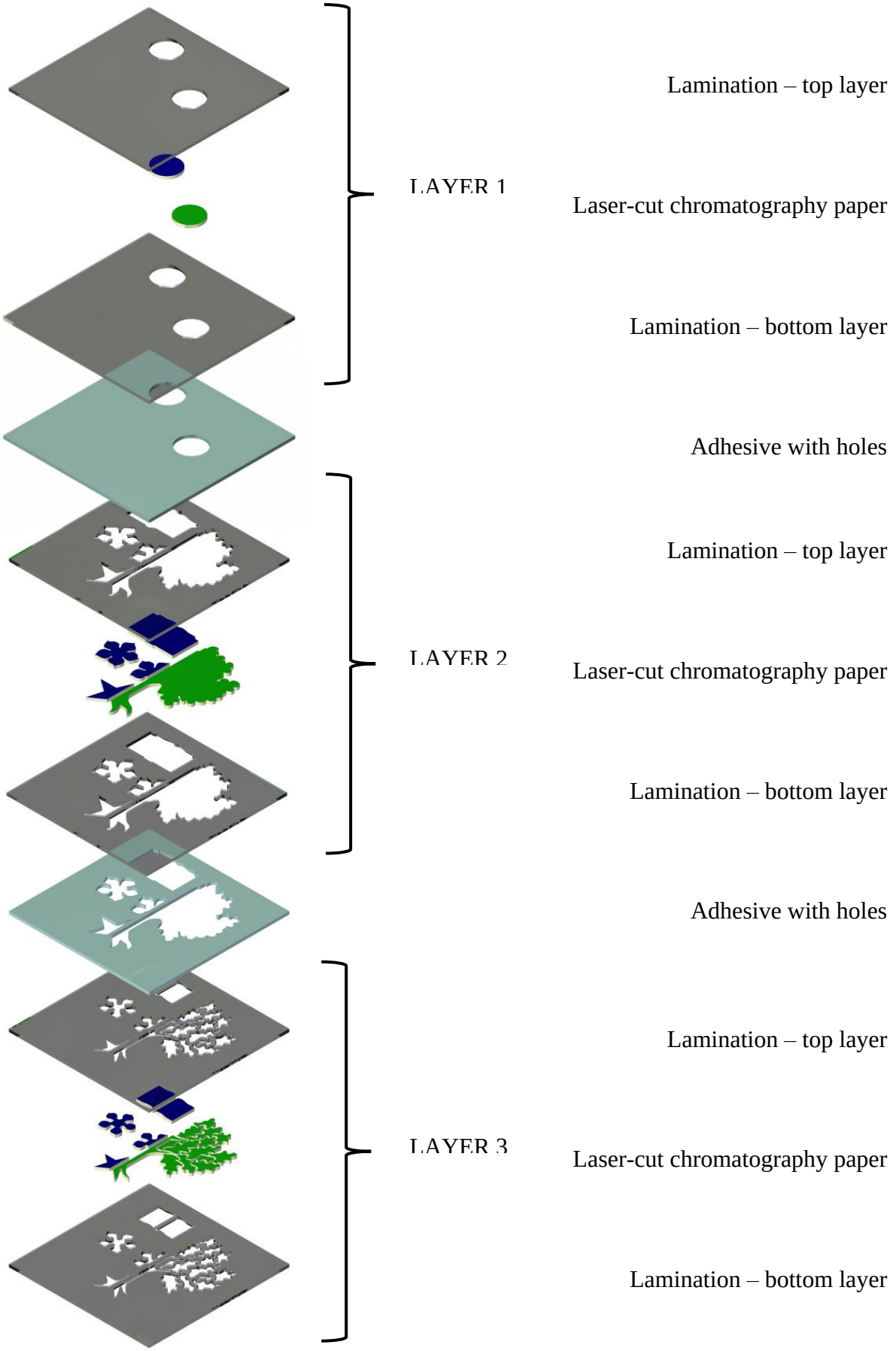



***Figure 12**: Design 2 – Heriot-Watt University's shield-of-arms*

### 4.3 Design 3 – Modified Paper Microzone Plate

This is a two dimensional device modelled based on the 96-well plate mentioned in the Literature Review section. The design was modified to include four test zones in each zone. Each test zone can contain a different reagent. The entire device measures 168mm × 72mm. To ease in the manual alignment of paper in lamination pouch, the paper zones were modelled to be interconnected by channels. The channels can then be cut after lamination to disconnect the zones, making them independent and preventing fluid flow from one layer to another. Holes were cut in the lamination such that the inlet and test zones are exposed. In this way, reagents can be added to the test zones after lamination, preventing their denaturation during hot lamination. This design was considered as a large number of tests can be conducted using a small volume of liquid and 96-well plates are widely used in ELISA, making this design compatible with existing testing equipment and procedures. **Figure 13** below shows the exploded and front views of the design. See Appendix A.iii: Design  for engineering drawings of the design.

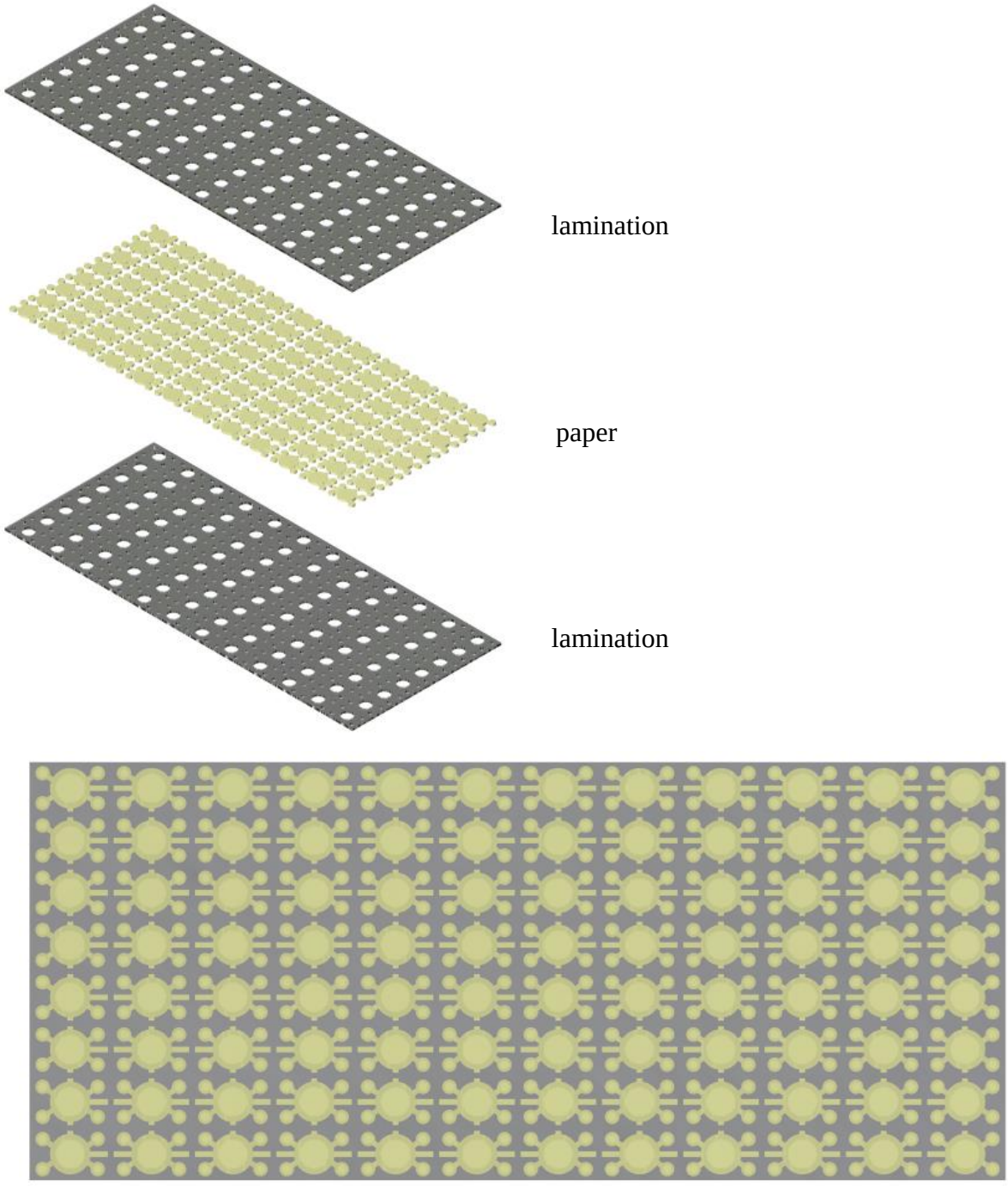



***Figure 13**: Design 3 – Modified paper microzone plate*

# 5 Fabrication and Testing Procedures

## 5.1 Set-up of Graphics

The sketches of the design can be done using CAD software or graphics editors. In this project, the designs were sketched and modelled in three dimensions using PTC Creo Parametric 3.0, which were then made into two-dimensional engineering drawings. The drawing files were converted into the DFX file format (.dfx) or DWG file format (.dwg) from the Creo default of DRW (.drw). These file formats are compatible with CorelDRAW, a graphics editing software which sends the print job to the laser software. However, the files could not be opened using the graphics editor installed in the computer connected to the laser cutter had an outdated version of the software (CorelDRAW X3). The files were opened using the latest version of the software (CorelDRAW X7) installed on another computer and converted to the CDR format (.cdr), that is compatible with X3.

The X3 .cdr files were copied to a USB flash drive and opened on the computer, shown in **Figure 14** below. The dimensions of the drawing are set on the toolbar. The different components can be set to have a different outline colour to ease the laser cutting process. This is done by dragging a box over the component to highlight it and double-clicking on the colour box at the bottom right corner of the interface which opens the *Outline Pen* window.

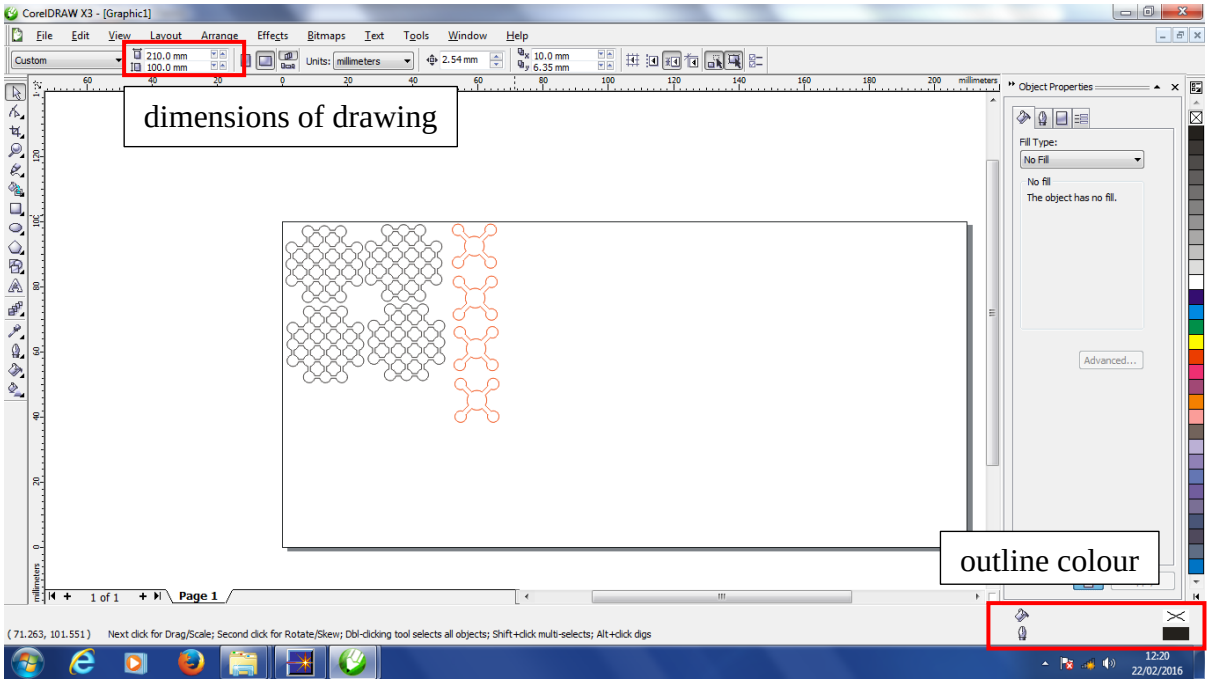



***Figure 14**:Graphics editor (CorelDRAW X3) interface*

Once the drawing is ready to be sent to the laser cutter, navigate to *File* > *Print*, which opens the *Print* window, shown in **Figure 15** below. In the *General* tab, ensure *Name* under *Destination* is set to the laser cutter (TrotecEngraver v9.4.2). Click on *Properties*, then click on the *Print* tab. Ensure *Minimise to Jobsize* is unchecked. Set *Width* and *Height* to equal the dimensions of the drawing set earlier. Click *OK* and *Print* in the *Print* window.

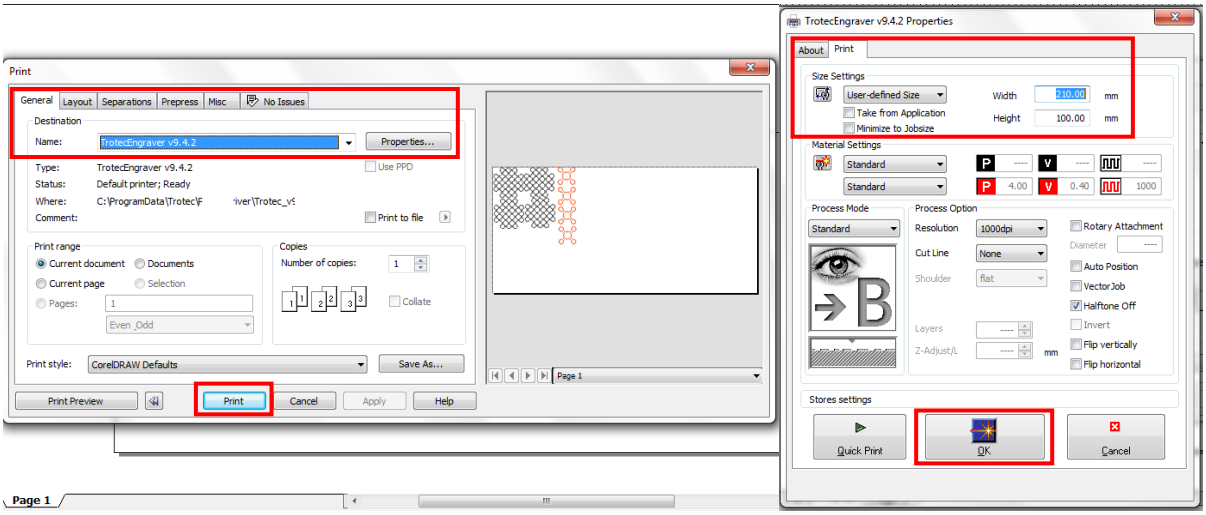



***Figure 15**: Setting print properties for the laser cutter on the graphics editor*

## 5.2 Laser Cutting

Switch on the laser cutter (TROTEC Speedy 300) (**Figure 16**). Align the material that needs to be cut, either chromatography paper or lamination pouch, on the base of the laser cutter. Place a calibration tool on the side of the laser pointer and using the control buttons, move the pointer to be above the material and slowly push the up button to move the base upwards until the calibration tool falls off, indicating the calibration is complete. Remove the calibration tool and close the lid of the machine.

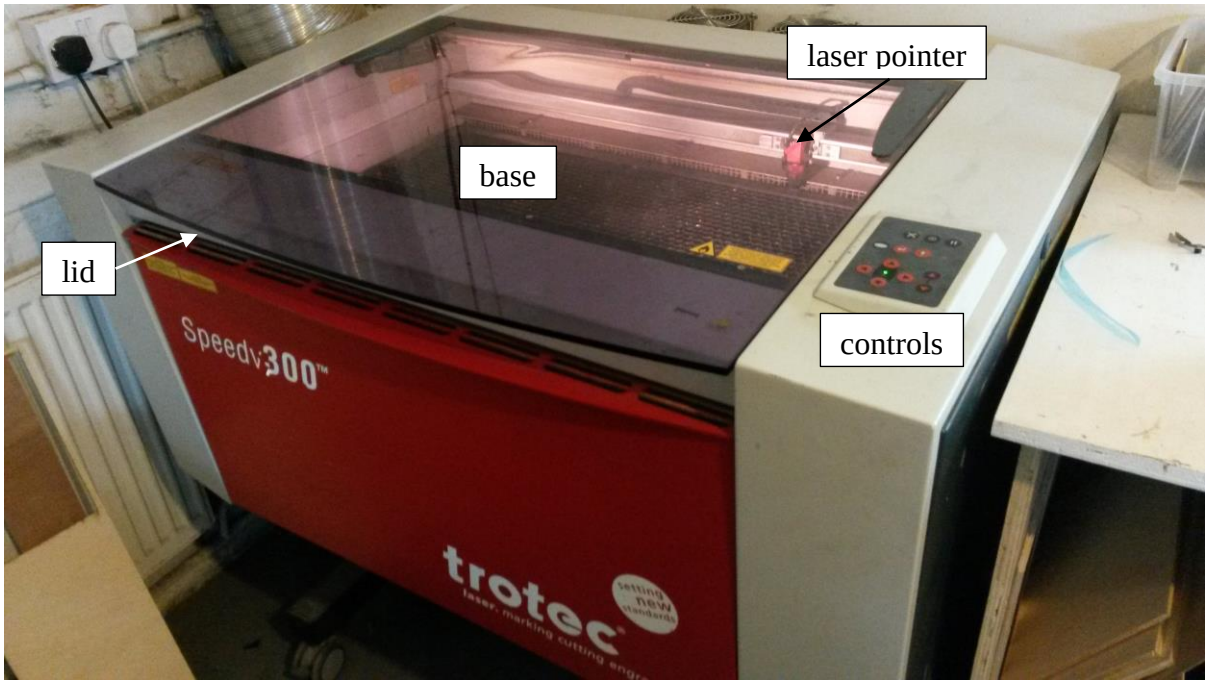



***Figure 16**: Labelled photograph of laser cutter (TROTEC Speedy 300)*
*Adapted from [38]*

Open the laser software (TROTEC JobControl 9.4.2), shown in **Figure 17** below. First, connect the computer to the laser cutter using the socket icon in the bottom of the menu on the right. Use the controls of the laser machine to move the pointer until a cursor appears on the canvas.

The cursor indicates the position of the laser pointer. Click on the material templates icon in the top menu.

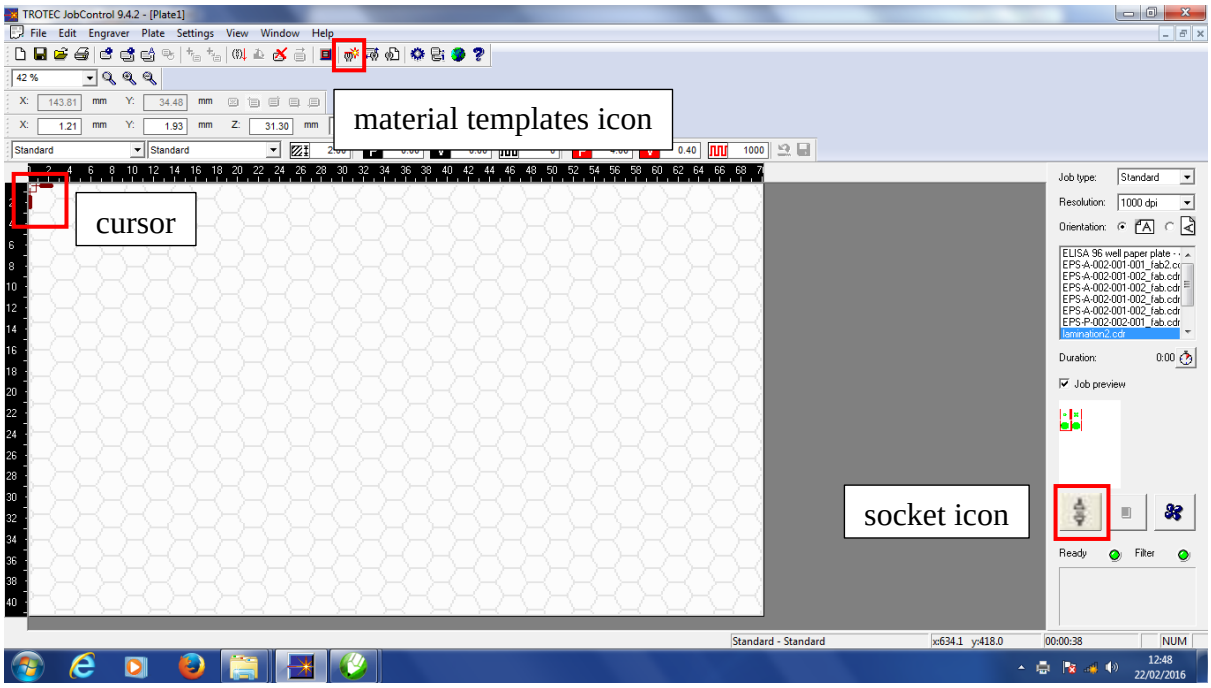



***Figure 17**: Laser software (TROTEC JobControl 9.4 2) interface*

On the *Material Templates* window (**Figure 18**), under *Settings*, click *More*. Set the colours to be cut corresponding to the outline colours of the drawings earlier to *Cut* and the rest to *Skip*. Use the following settings: *Power* 4%, *Speed* 0.40, PPI/Hz 1000, *Passes* 1 (for paper) and 2 (for lamination), *Air assist* 1, *Correction* 0, *Z-Offset* 0.00mm; and click *Save*.

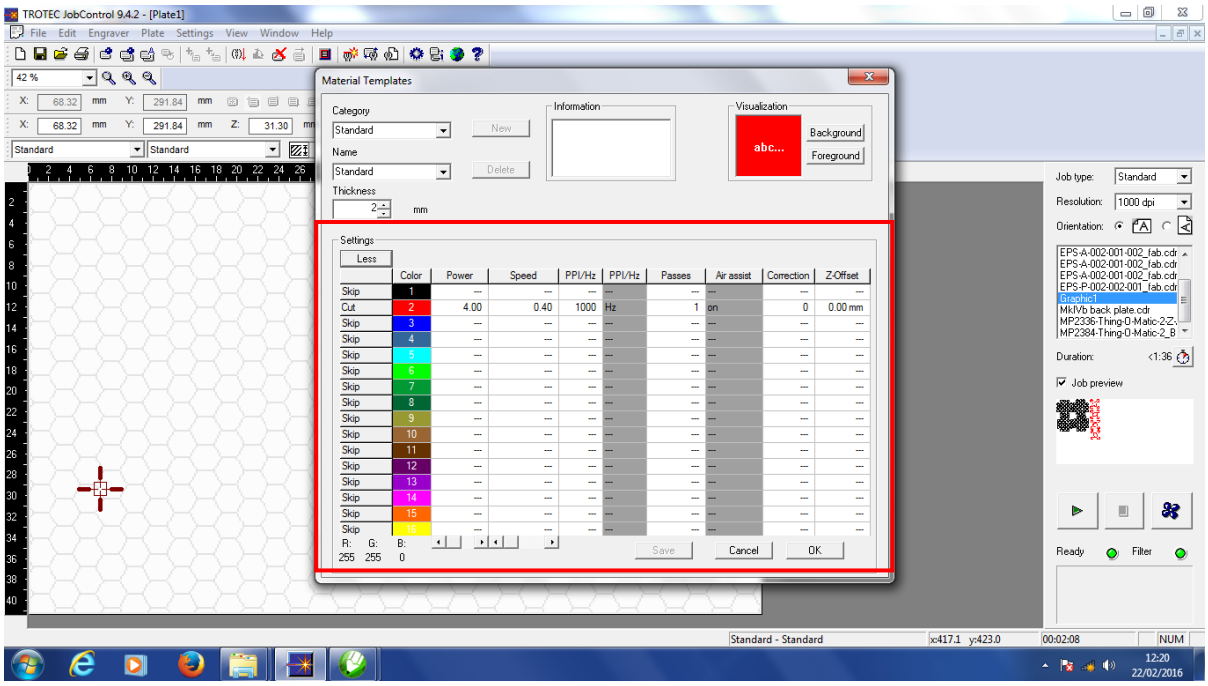



***Figure 18**: Material template window on laser software*

The printed job should appear in the menu on the right. Click on it and drag it to the canvas and snap its top left corner to the cursor (**Figure 19**). The job will be highlighted in grey and will be in the dimensions of the drawing. Align the material in the laser cutter to correspond to the position of the job. Click on the play icon in the bottom of the right menu to start the laser cutting process.

NOTE: Use low power to prevent paper or lamination from burning. Avoid direct eye contact with laser while in operation.

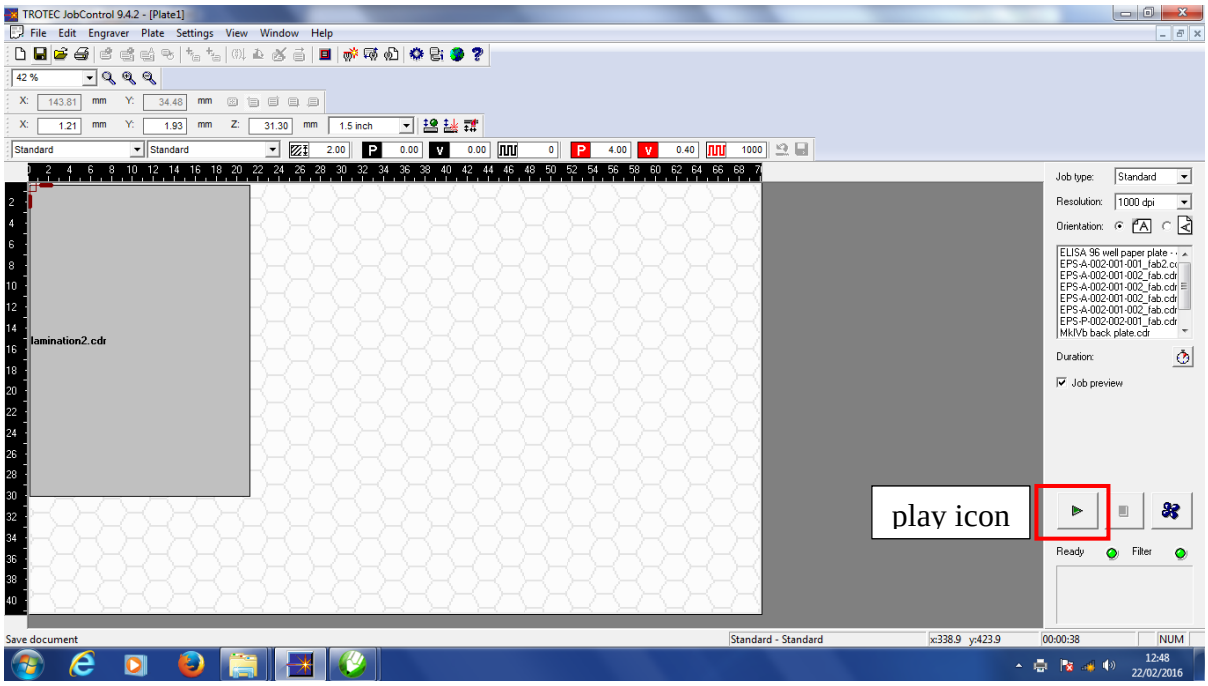



***Figure 19**: Job highlighted in grey ready to be cut*

## 5.3 Lamination

Once the paper has been cut in shape and the holes have been cut in the lamination pouch, the paper is aligned in the pouch as explained in the Chosen Fabrication Method section. This is then laminated using the hot roll laminator (GBC Catena 35) shown in **Figure 20**.

Before use, switch on the laminator and use the controls in **Figure 21** to set the following: *Temperature* 110°C, *Speed* 5, *Roller pressure* 1mm. The laminator is ready to use once the *Ready* LED on the controls flashes in green. Place the edge of the sheet in between the hot roll and the base and press and hold *Run* to start laminating. Release the button once the whole sheet has finished laminating. If the laminated sheet is not flat, run it through the laminator again in reverse.

NOTE: Avoid touching the hot laminator rolls and keep clothing and hair away from the machine during operation.


***Figure 20**: Hot roll laminator (GBC Catena 35)*

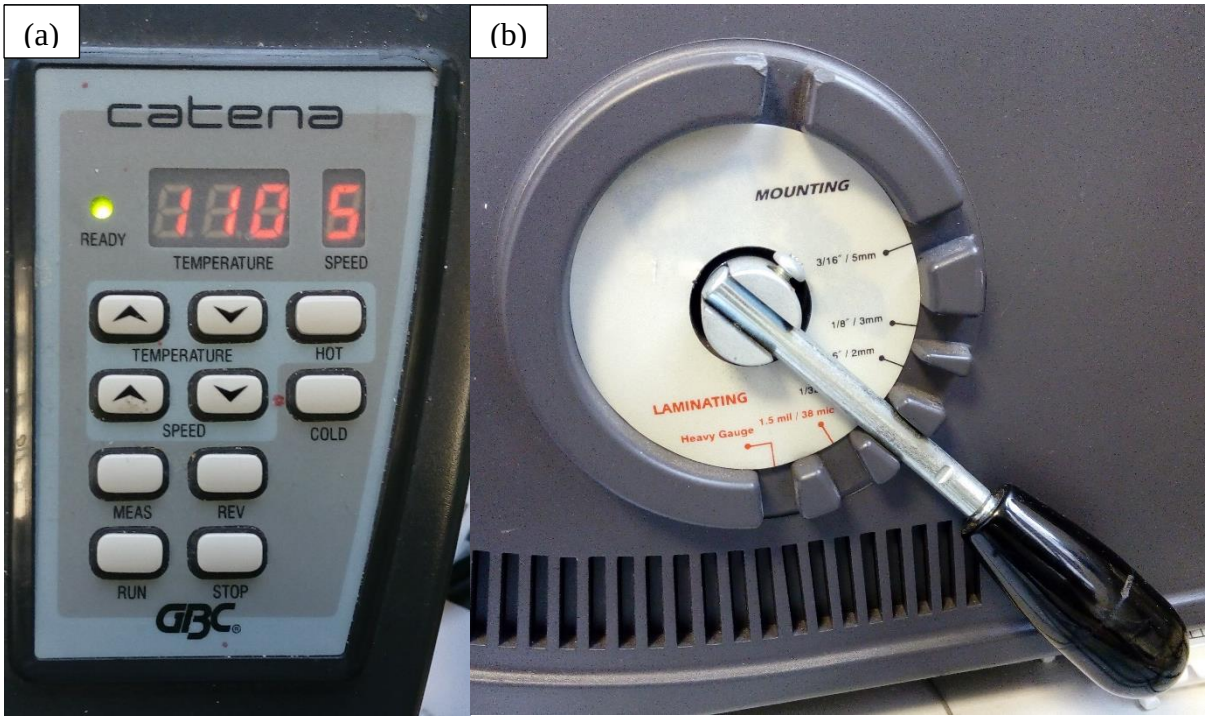



***Figure 21**: Laminator controls*
*consisting of (a) run, reverse and stop buttons and temperature and speed settings, and (b) roller pressure handle*

### 5.4 Assembly

After lamination, cut the layers into squares using scissors. For three-dimensional devices, apply adhesive on the bottom side of each layer except the final and stack the layers in order carefully on one another, ensuring there is vertical alignment of the hydrophilic areas. Apply pressure to the assembled device to ensure secure attachment.

### 5.5 Testing

A micropipette (DragonMed 20-200µl) was used in testing (**Figure 22**). The test fluid used was food colouring (see Appendix B.iv: Food Colouring for data sheet) diluted 1:1 with tap water. Food colouring was used due to the contrast it provides with white paper and as it is not hazardous. The main purpose is to test whether fluid is able to flow vertically from the top layer to the bottom of the device. Animal disease testing was not done as biohazards are involved and since the university does not have the training and facilities required for such tests, it has to be conducted off campus while being supervised.

Fit the pipette with the appropriate pipette tip and extract the volume of food colouring required to test the device. Apply the fluid fully on an inlet of the device as shown in **Figure 23** and start a stopwatch. Observe the fluid flow and record the time taken for each layer to fully wet and for the fluid to flow from one layer to another.

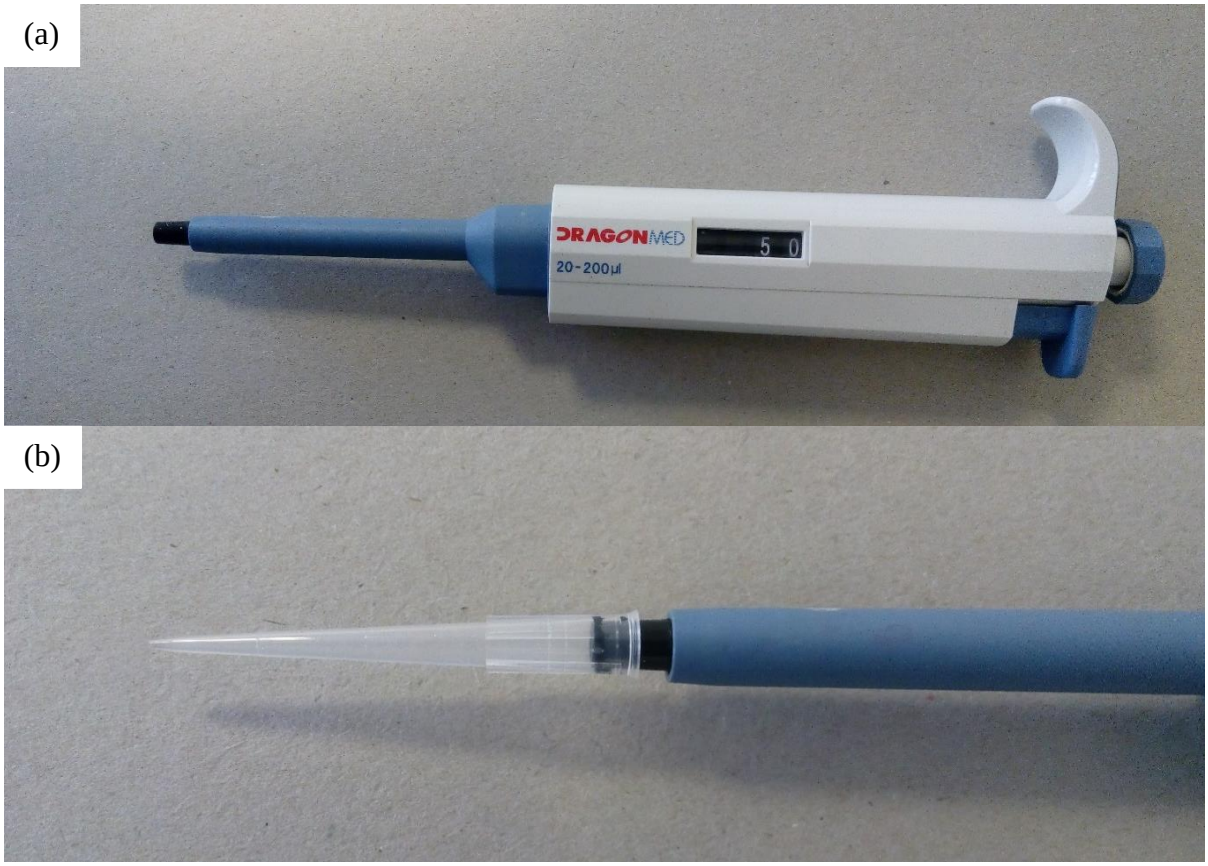



***Figure 22**: Micropipette (DragonMed 20-200µl)*
*(a) showing volume of liquid used in tests, and (b) with pipette tip*

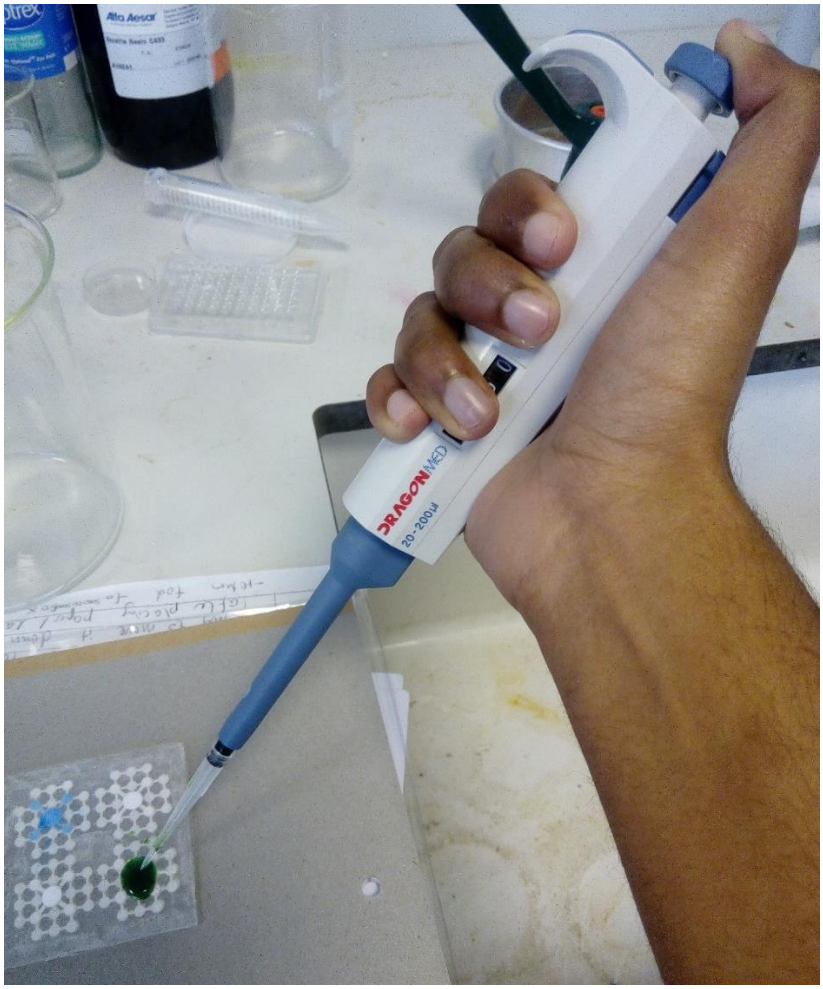



***Figure 23**: Application of test fluid in the inlet*

# 6 Results and Discussion

## 6.1 Design 1

### 6.1.1 First Prototype

The first prototype was made as detailed in the above sections, except office double-sided tape was used as the adhesive and added to only the edges of layers, and is shown in **Figure 24**.

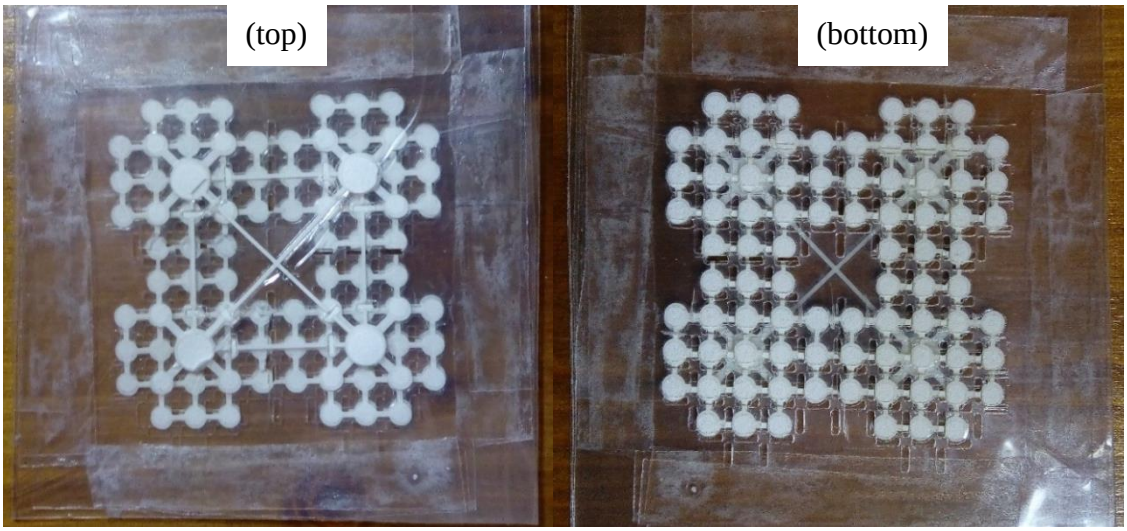



***Figure 24**: Top and bottom views of Prototype 1 of Design 1 before testing*

50µl of green food colouring was applied to the top left inlet and 50µl of blue food colouring was applied to the top right inlet. The layers were separated after testing and the scanned images are shown in **Figure 25**. Since adhesive was only applied at the edges of the layers, large gaps existed between the layers, causing the excessive fluid to run in between layers instead of travelling vertically from the inlet to the zones below. As shown by the images, this resulted in cross-contamination of adjacent zones in the first and second layer.

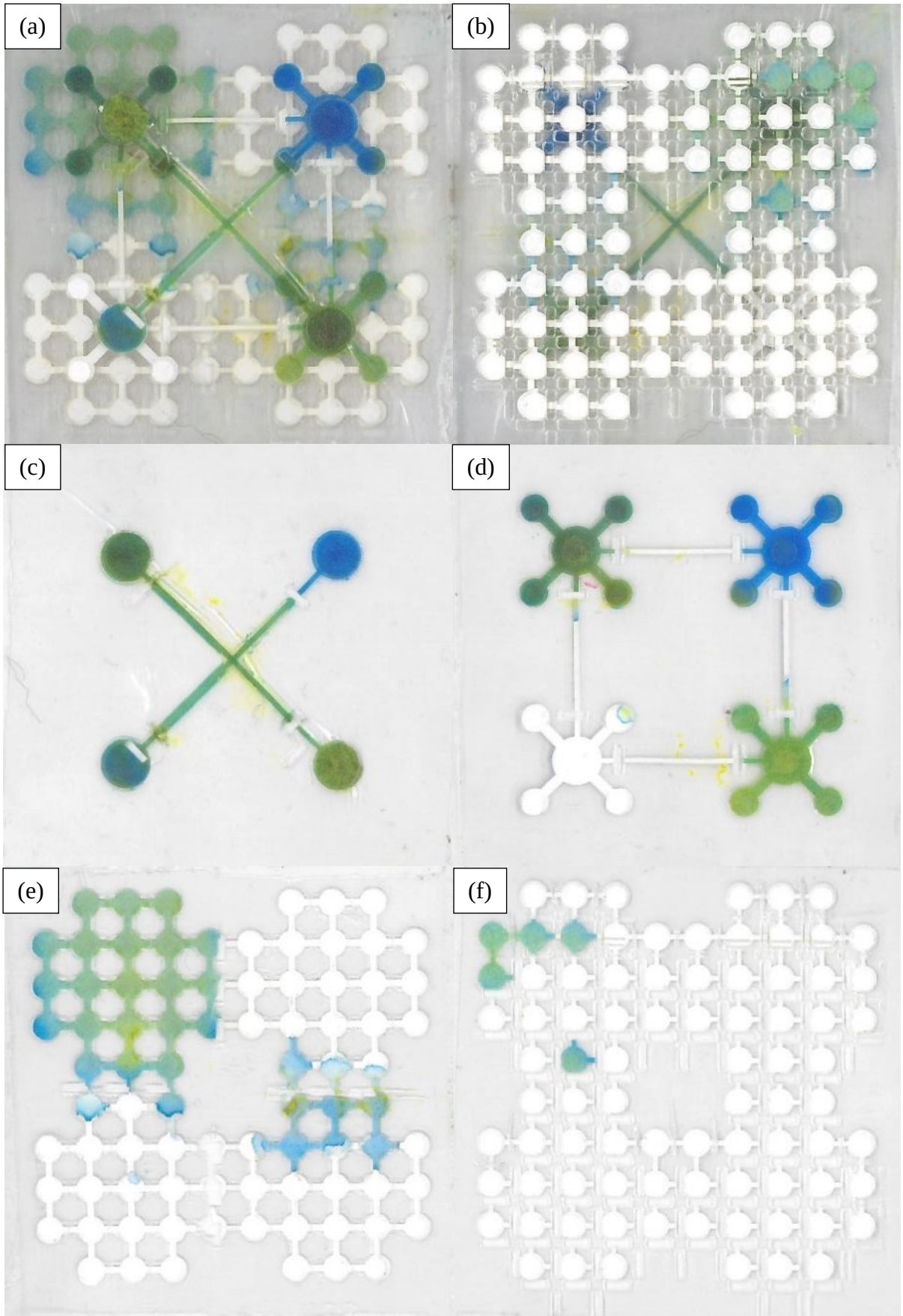



***Figure 25**: Prototype 1 of Design 1 after testing*
*showing (a) top view, (b) bottom view, (c) layer 1, (d) layer 2, (e) layer 3 and (f) layer 4*

### 6.1.2 Second Prototype

A second prototype was made using a simplified version of Design 1, featuring a quarter of the design with one inlet. This was done to save the amount of materials used as well as reduce the time taken to manufacture and test. The device was assembled in a similar way to Prototype 1 but using acrylic adhesive transfer tape (3M 9471 300LSE Adhesive Transfer Tape) (see Appendix B.iii: Adhesive Transfer Tape for data sheet) which has a higher bond strength which is maintained even after exposure to water and is thinner. [39] This aimed to reduce the gap between layers, secure the device strongly and prevent fluid from leaking through the adhesive. An extra layer of paper was also added in between the layers to increase contact. This was tested with 50µl of blue food colouring. However, as shown by the before and after testing images in **Figure 26**, although the adhesive covered majority of the hydrophobic areas and reduced the gaps, the fluid still diffused out of the paper and into the smaller gaps that exist.

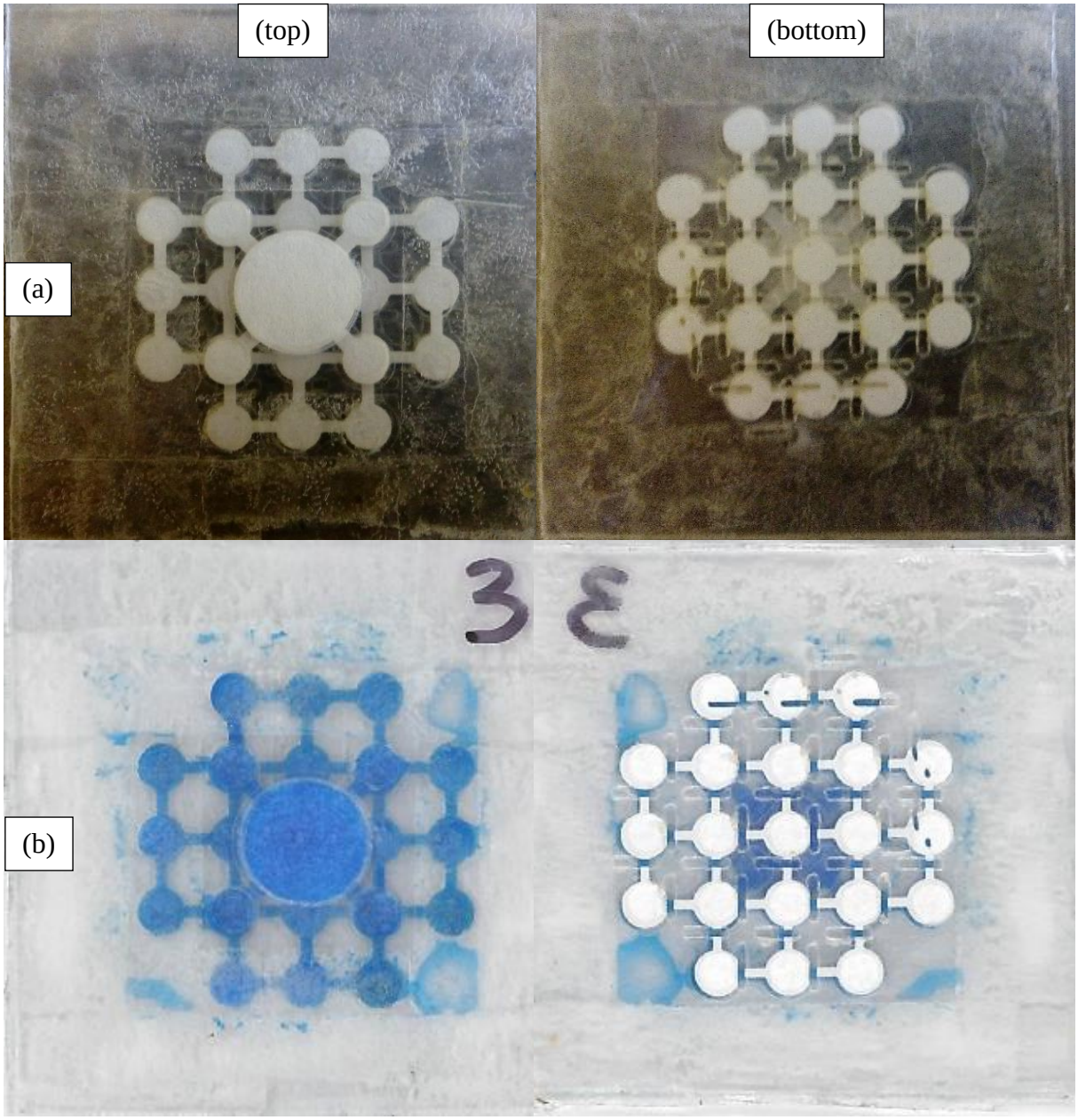



***Figure 26**: Top and bottom views of Prototype 2 of Design 1*
*(a) before testing and (b) after testing*

### 6.1.3 Further Prototypes

For the subsequent prototypes, the adhesive was applied to the lamination sheet prior to cutting holes, as shown in **Figure 27**. This is to ensure the adhesive covers the entire hydrophobic area of the layers. The holes cut would provide contact points between adjacent hydrophilic areas only. The adhesive has a temperature resistance of 148°C [39], therefore it still retains its bond strength after hot lamination. The backing is only peeled during the assembly process. Due to the high bond strength and coverage of the adhesive, the liquid would only be able to flow from the paper in one layer to the next. This would also even out the thickness distribution of the device as shown in the calculations below.

Thickness of one sheet of chromatography paper, $T_P = 0.18\text{mm}$ [18]

Thickness of one lamination pouch, $T_L = 0.075 \times 2 = 0.15\text{mm}$ [40]

Thickness of adhesive (without backing) $T_A = 0.058\text{mm}$ [39]

Thickness of two layers without paper, $T_1 = 2T_L + T_A = 2 \times 0.15 + 0.058 = 0.358\text{mm}$

Thickness of two layers of paper, $T_2 = 2T_P = 2 \times 0.180 = 0.36\text{mm}$ $(\approx T_1)$

***Equation 1**: Thickness distribution calculation for three-dimensional devices*

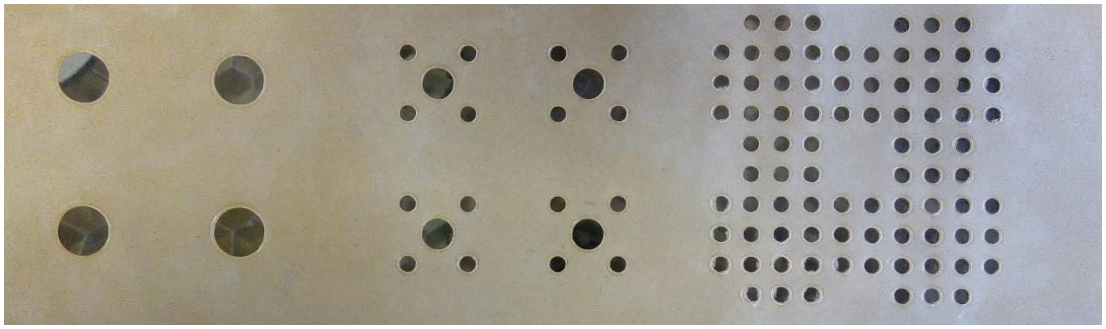


***Figure 27**: Photograph of lamination backed with adhesive after laser cutting of holes*

A third prototype was made using this method and was tested using 50µl of blue food colouring. The before and after testing images are shown in **Figure 28**. No fluid leaks were detected and the fluid successfully flowed to the final layer. Two more similar prototypes (**Figure 29** and **Figure 30**) and a full scale prototype (**Figure 31**) were made and tested. Again, the fluid did not leak, proving that this method of adhesive application prevents the formation of gaps between layers and increases contact between the hydrophilic areas of adjacent layers. The time taken and observations noted when testing prototypes 3, 4 and 5 are recorded in **Table 1**, **Table 2** and **Table 3** respectively. It can be seen that within 18 minutes, 50µl of fluid fully wicks into the paper, showing that the tests are rapid.

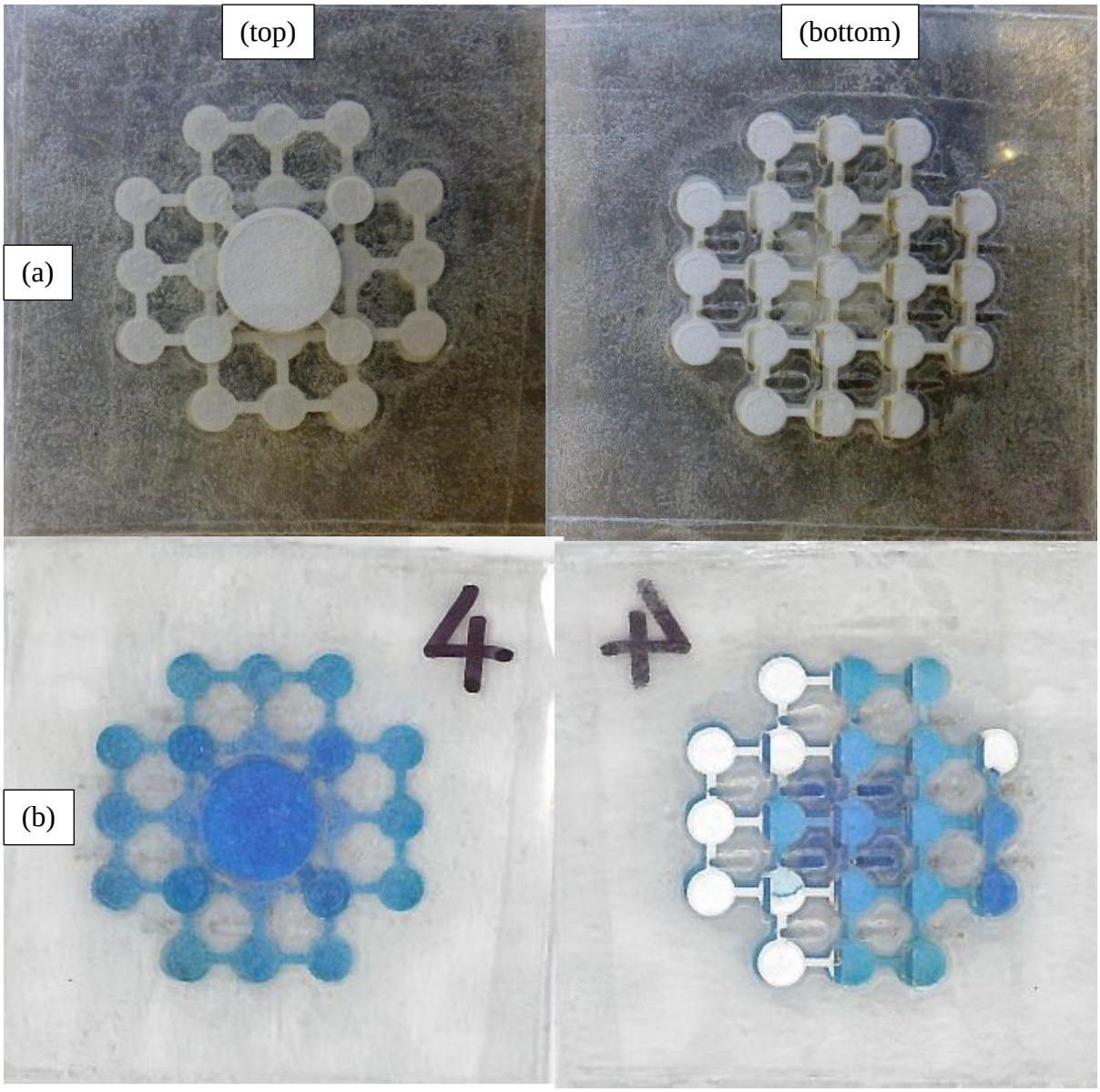



***Figure 28**: Top and bottom views of Prototype 3 of Design 1*
*(a) before testing and (b) after testing*

| **Time, t** | **Observation** |
|---|---|
| 0min | 50µl of food colouring added to inlet |
| >1min | Colour observed in second layer |
| ~1min 30sec | Colour visible in third layer |
| ~2min | Second layer fully wet |
| ~17min 30sec | Third layer fully wet and 13 test zones coloured in fourth layer |

***Table 1**: Testing time and observations for Prototype 3 of Design 1*

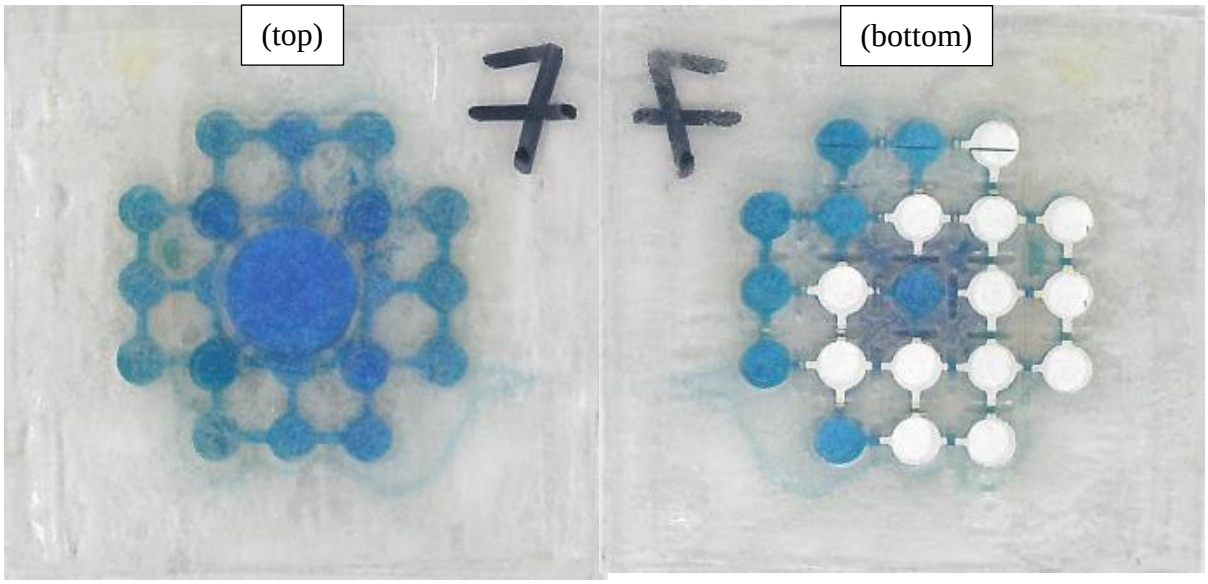



***Figure 29**: Top and bottom views of Prototype 4 of Design 1 after testing*

| **Time, t** | **Observation** |
|---|---|
| 0min | 50µl of blue food colouring added to inlet |
| ~0min 30sec | Colouring visible in second layer |
| ~2min | Second layer fully wet and colour visible in the centre test zone in the fourth layer |
| ~15min | Third layer fully wet and 8 test zones coloured in fourth layer |

***Table 2**: Testing time and observations for Prototype 4 of Design 1*

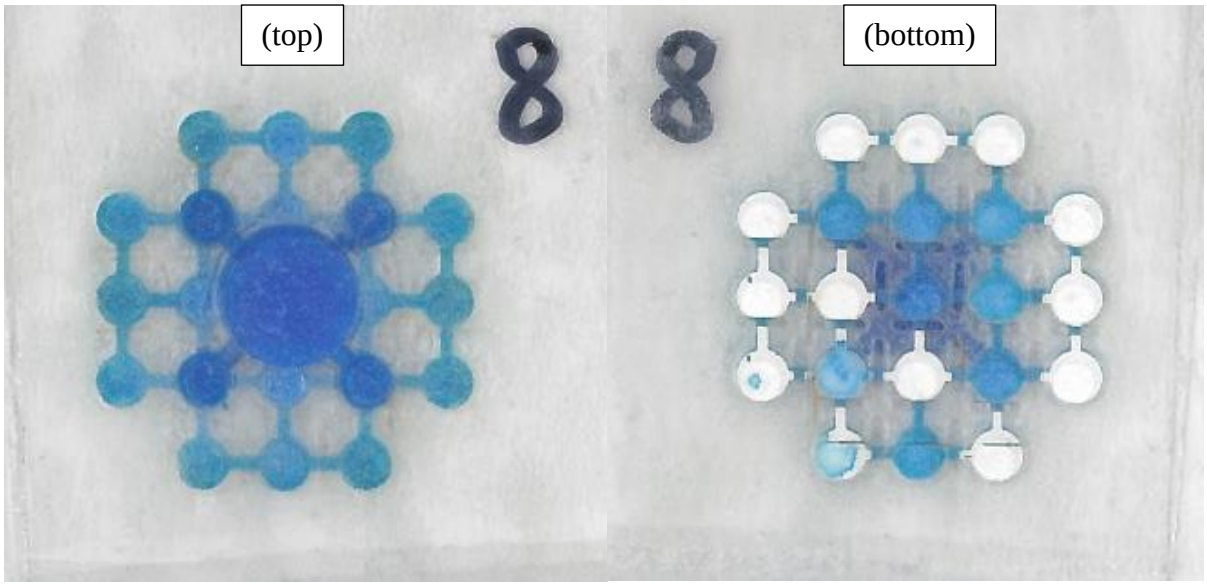



***Figure 30**: Top and bottom views of Prototype 5 of Design 1 after testing*

| **Time, t** | **Observation** |
|---|---|
| 0min | 50µl of blue food colouring added to inlet |
| ~1min | Colouring visible in second layer |
| ~2min | Second layer fully wet |
| ~16min | Third layer fully wet and 8 test zones coloured in fourth layer |

***Table 3**: Testing time and observations for Prototype 5 of Design 1*

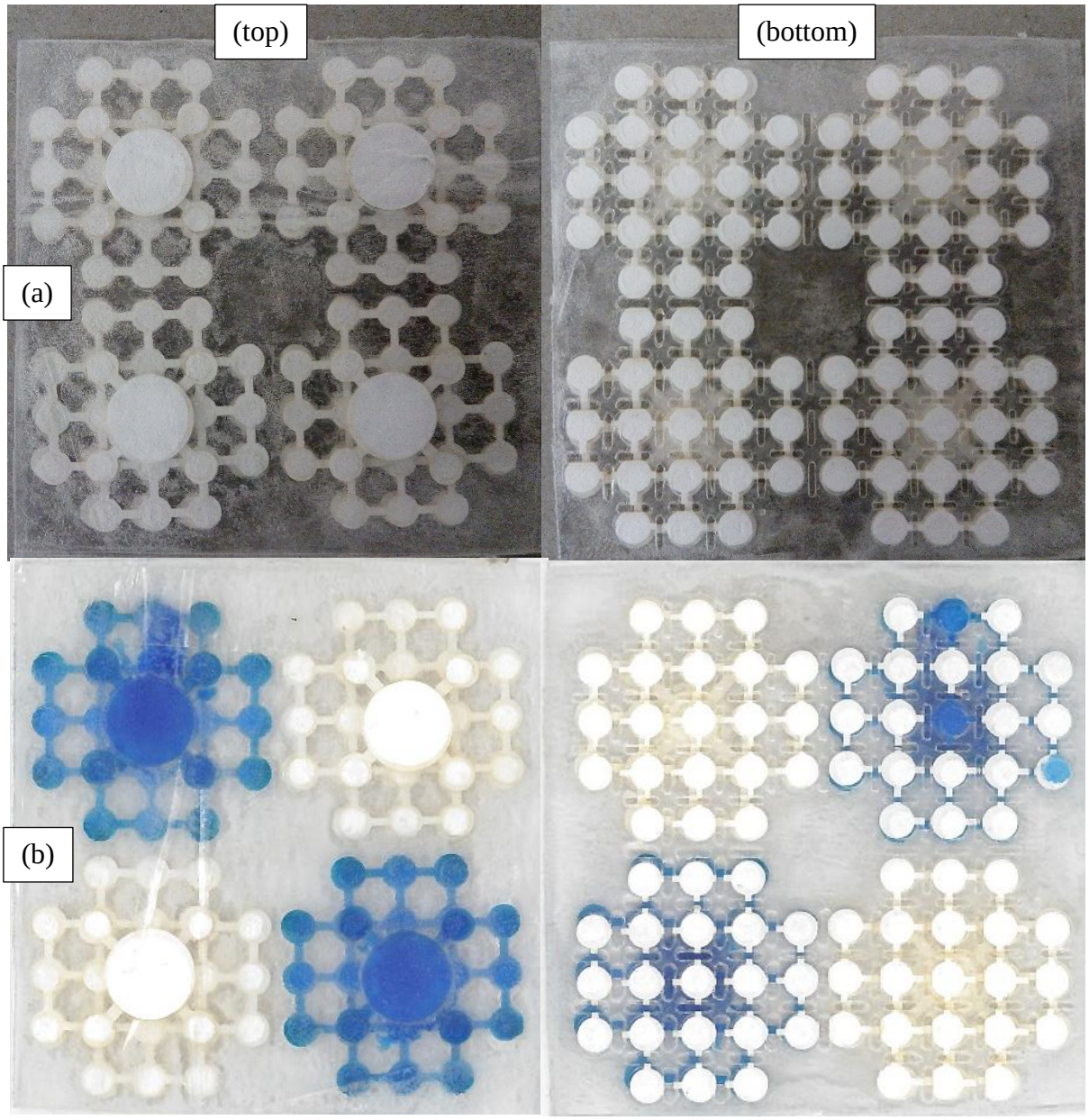



***Figure 31**:Top and bottom views of Prototype 6 of Design 1*
*(a) before testing and (b) after testing*

### 6.2 Design 2

A prototype is based on Design 2 was fabricated using the same method and was tested using 100µl of green and blue food colouring. The images of the device before and after testing in **Figure 32** show that the fluid travelled to all three layers without leaks and fully wetted the tree-shaped test zones. This confirms that it is possible to fabricate devices with complicated features through this method and produce a working prototype. However, due to the irregularities in shape of the hydrophilic areas and the difficulty involved in the manual alignment of the small paper shapes in the lamination pouch which is time consuming, this device is not ideal for biomedical applications and quantitative testing.

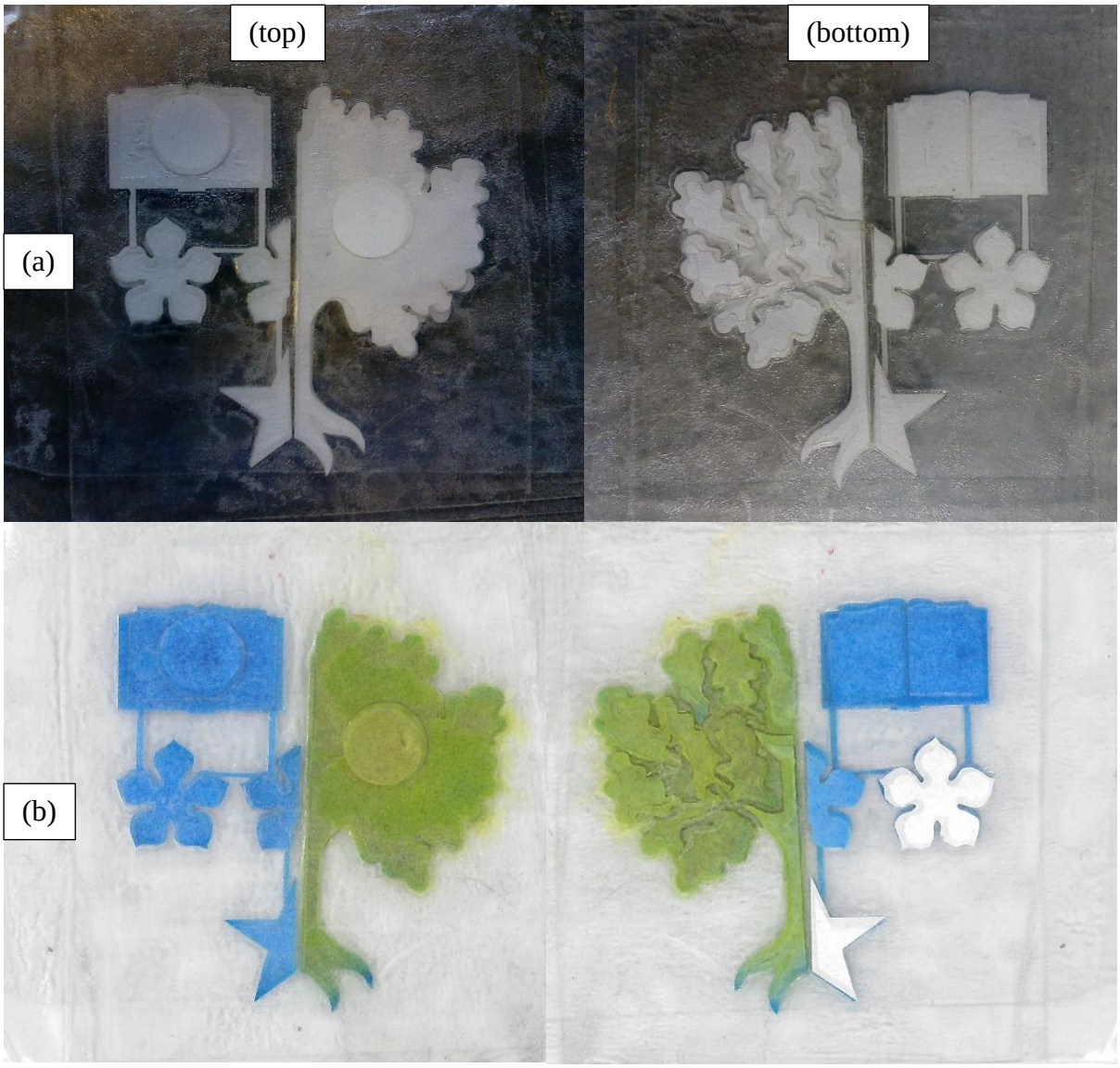



***Figure 32**: Top and bottom views of prototype of Design 2 (a) before testing and (b) after testing*

### 6.3 Design 3

A device based on Design 3 was fabricated and tested using an eight-channel micropipette. 10µl of blue or red food colouring was added to alternating inlets and the results are shown in **Figure 33**. Due to rapid concentration of the food colouring, it can be seen that the intensity of colour is higher in the inlets compared to the other areas, making it more sensitive to detection. Using the histogram function of Adobe Photoshop, the mean intensities of the blue and red spots and the control zones were obtained and the results are shown in **Table 4**. However, a comparison cannot be made as the testing only involved food colouring of a single concentration.

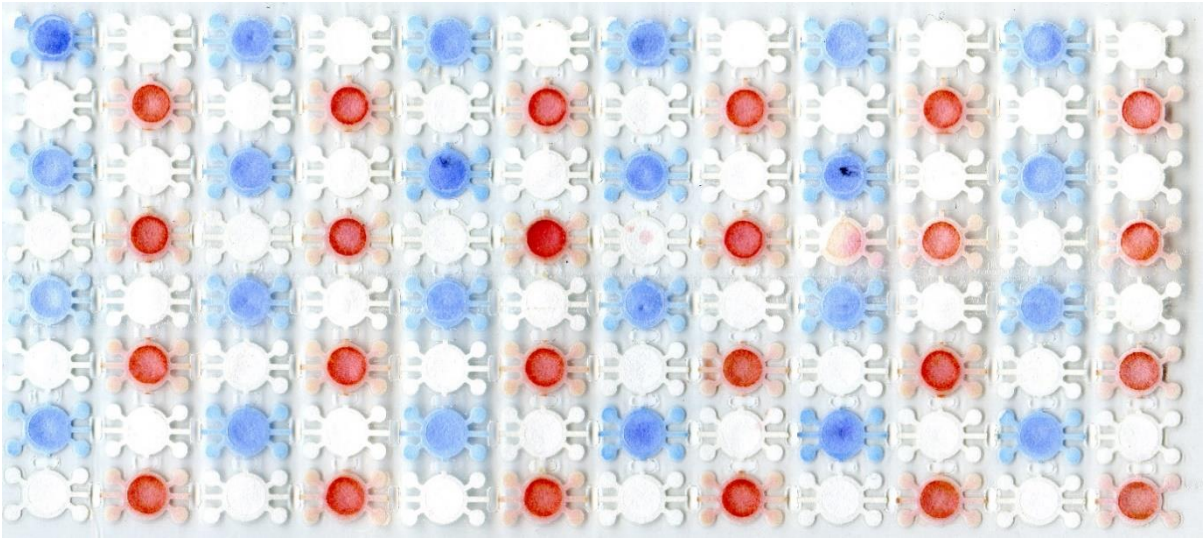


***Figure 33**: Prototype of Design 3 after testing*

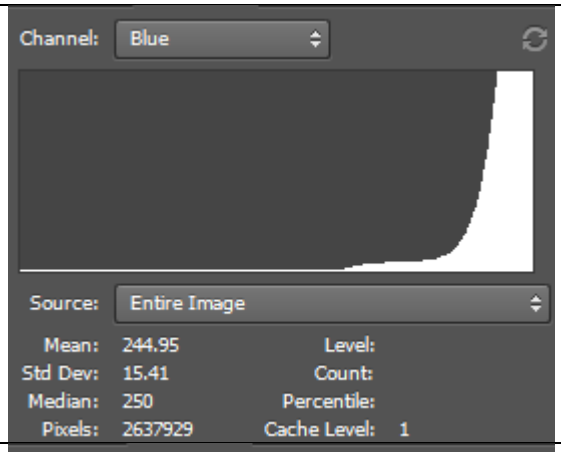
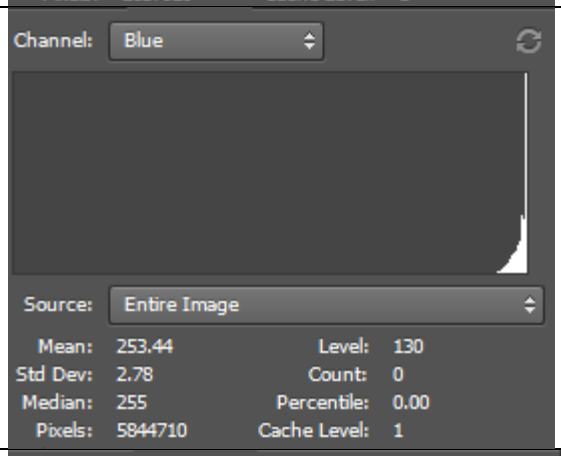
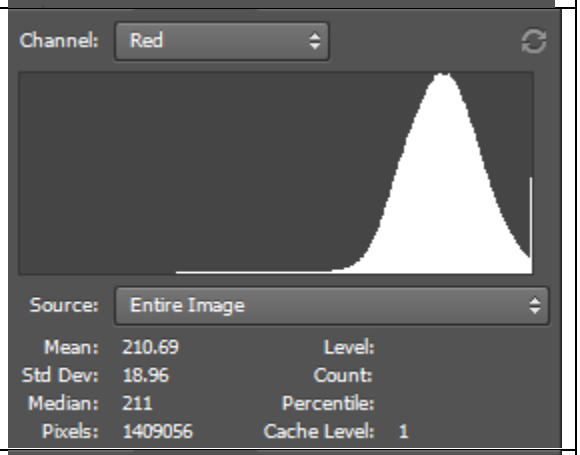
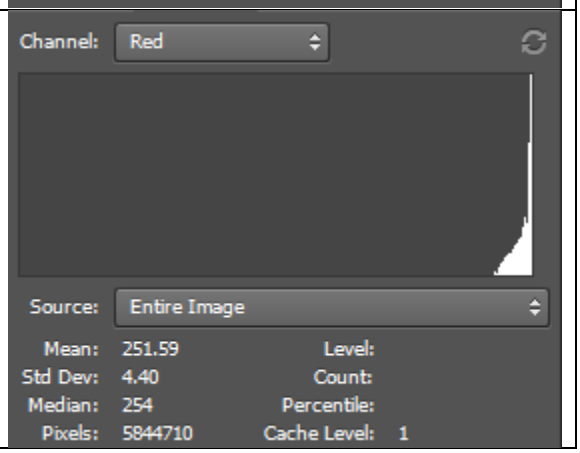
| | |
|---|---|
| Blue colour intensity of zones tested with blue food colouring |  |
| Blue colour intensity of zones not tested with any colouring (plain white paper) |  |
| Red colour intensity of zones tested with red food colouring |  |
| Red colour intensity of zones not tested with any colouring (plain white paper) |  |

***Table 4**: Measuring the intensity of zones, both tested and untested, using the histogram function on Adobe Photoshop*

# 7 Estimated Cost

From online retail prices of materials, an estimate for the cost of materials per $m^2$ of device was calculated and recorded in **Table 5**. This is higher than the cost of materials if the wax printing method was used [16] as the paper cutting method uses more materials and produces more waste as the elements are cut in shape, and a more expensive adhesive is used.

| **Material** | **Unit** | **Unit Cost (£)** | **Total Area ($m^2$)** | **Cost per $m^2$ (£)** |
|---|---|---|---|---|
| Chromatography paper [18] | 100 20cm×20cm sheets | 36.57 | 6.237 | 1.6001 |
| Lamination pouch [40] | 100 A4 (210mm×297mm) sheets | 9.98 | 4.000 | 9.1425 |
| | | | **Total (£)** | **10.7426** |
| Adhesive [41] | 305mm×55m roll | 187.12 | 16.775 | 11.1547 |
| | | | **Total (£)** | **21.8973** |

***Table 5**: Estimated cost of materials per square meter of a prototype based on retail price of materials*

From this estimate, a four-layer device measuring 50mm×50mm would cost £0.219 and the two-dimensional paper microzone plate (with no adhesive) would cost £0.1299.

# 8 Future Work

In the future, animal disease testing can be done off campus using prototypes based on Design 1 and Design 3. The first step is the addition of reagents in the test zones. For protein assays, this would be tetrabromophenol blue solution while for glucose assays, the reagent is glucose oxidase. Next, a buffer solution is prepared for the assay. An example of a protein assay is bovine serum albumin and an example of a glucose assay is glucose solution. [17]

A number of qualitative tests should be conducted in order to test the specificity and sensitivity of the devices. Sensitivity is the measure of the number of true positives, while specificity measures the number of true negatives. The more specific and sensitive the device, the more reliable and accurate it is.

Three-dimensional paper based microfluidic devices have the potential to filter unwanted substances from a fluid sample such as protein and blood cells in blood and only allowing serum to the test zones. This device uses paper with a pore size of 11μm [37] which is large enough for a red blood cell with a diameter and thickness of approximately 8μm and 2μm respectively [42] to squeeze through. In addition to using paper with a smaller pore size, antibodies for the agglutination of the cells can be added to the middle layers, [3] which will cause the cells to clot and unable to diffuse to the adjacent layer.

Through testing, it was found that 50μl of fluid was insufficient to fully wet the device based on Design 1. The design can be simplified to include three layers with the current second layer removed so that a smaller volume of liquid is required. Although paper is a porous medium and the pore size is known, it is difficult to calculate the exact volume of liquid required to wet the paper through correlations. [22] The ideal volume can be found through testing by gradually increasing the volume of fluid until all test zones are fully wet. The volume would differ for different fluids due to their viscosity, density, concentration and presence of other particles and substances.

From measuring the intensity of the inlet versus the intensity of test zones, the effective concentration of the fluid at the test zones can be found. This is important when the intensity of zones is measured to produce calibration curves and to identify the flow pattern of a fluid in paper. Further prototypes should be fabricated and tested with a range of concentrations of dye solutions as well as buffer solutions to construct calibration curves for the intensity of colour of tests.

Two-dimensional devices made through the paper cutting and lamination method may be more flexible as there is no support for the paper. After the addition of reagents, the bottom of the device should be sealed with cold lamination or plastic to increase its tensile strength as well as prevent contamination of reagents.

# 9 Conclusion

In conclusion, this project aimed to fabricate paper-based microfluidic devices suited for animal disease diagnostics especially for livestock. As early disease diagnosis is an important step in DIVA and preventing further spread of diseases, it is crucial that the devices are able to conduct multiple tests simultaneously using small sample volumes and have high rapidity. From test results, the devices based on Design 1 and Design 3 were both found to produce results that match the criteria.

Another aim of this project is to solve the key problem faced by researchers working on three-dimensional devices, which is maintaining contact points between hydrophilic areas of adjacent layers for fluid to flow vertically from the inlet on the top layer to the test zones on the bottom. Through fabrication and testing of six prototypes, the fabrication method involving paper cutting and lamination with adhesive covering the entire hydrophobic areas between layers produced devices with even thicknesses that only allow fluid flow from one hydrophilic area to another.

These devices have simple fabrication methods where rapid prototyping can be conducted provided the fabrication equipment and materials are available. The devices are user-friendly and can be used by a person with no training in disease diagnostics with sufficient instructions provided. Therefore, the device can be made and stored and used during an emergency or an outbreak of a disease in a farm by workers to obtain quick results.

Animal disease testing has to be conducted initially using buffer solutions prior to in situ use to evaluate the sensitivity and specificity of the devices that show their performance. In addition, using known concentrations of assays to test a device allows for calibration curves to be constructed. When an assay of unknown concentration is tested, its intensity can be measured and the corresponding concentration can be found from the curve.

The estimated material cost for a three-dimensional device made using this fabrication method is approximately £21.90 per $m^2$, which is higher than that of wax printing method due to more materials and a more expensive adhesive used. From this estimate, a three-dimensional four-layer device measuring 50mm×50mm would cost £0.219 and the two-dimensional paper microzone plate (with no adhesive) would cost £0.1299, which are both reasonable for disposable one-time use diagnostics.

## Acknowledgements

I would like to thank my supervisor Dr. Will Wenmiao Shu, Reader in Microengineering at Heriot-Watt University, who proposed the project and has provided endless guidance on my work. This project would not have been made possible without the support of Valentina Busin, PhD candidate in Biomedical Microengineering at Heriot-Watt University, who has trained me in fabrication and packaging of paper-based microfluidic devices and supervised me while I used the laser laboratory at all times.

## References

[1] Whitesides Research Group, "Research > Microfluidics," Whitesides Research Group, 2015. [Online]. Available: http://gmwgroup.harvard.edu/research/index.php?page=19. [Accessed 30 November 2015].

[2] A. W. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas III, H. Sindi and G. M. Whitesides, "Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis," *Analytical Chemistry*, vol. 80, no. 10, pp. 3699-3707, 2008.

[3] A. K. Yetisen, M. S. Akram and C. R. Lowe, "Paper-based microfluidic point-of-care diagnostic devices," *Lab on a Chip,* vol. 13, no. 1, pp. 2210-2251, 2013.

[4] M. Bounpheng, "Agricultural Screening Tools," Institute for Infectious Animal Diseases, Texas A&M University, 2016. [Online]. Available: http://iiad.tamu.edu/tools/agricultural-screening-tools/. [Accessed 29 March 2016].

[5] A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, "Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices," *Analytical Chemistry*, vol. 82, no. 1, pp. 3-10, 2010.

[6] H. Kettler, K. White and S. Hawkes, "Mapping the landscape of diagnostics for sexually transmitted infections," World Health Organisation, Geneva, 2004.

[7] T. Aluwong and M. Bello, "Emerging diseases and implications for Millennium Development Goals in Africa by 2015 - an overview," *Veterinaria Italiana*, vol. 46, no. 2, pp. 137-145, 2010.

[8] World Health Organisation, "WHO global strategy for food safety : safer food for better health," World Health Organisation, Geneva, 2002.

[9] G. M. Whitesides, "A lab the size of a postage stamp," TEDxBoston, July 2009. [Online]. Available: http://www.ted.com/talks/george_whitesides_a_lab_the_size_of_a_postage_stamp. [Accessed 23 April 2016].

[10] J. Hassink and M. van Dijk, Farming for Health: Green-Care Farming Across Europe and the United States of America, Dordrecht: Springer, 2006.

[11] I. D. Robertson, P. J. Irwin, A. J. Lymbery and R. C. Thompson, "The role of companion animals in the emergence of parasitic zoonoses," *International Journal of Parasitology*, vol. 30, no. 12-13, pp. 1369-1377, 2000.

[12] World Health Organisation, "Foodborne disease outbreaks: Guidelines for investigation and control," World Health Organisation, Geneva, 2008.

[13] X. Li, D. R. Ballerini and W. Shen, "A perspective on paper-based microfluidics: Current status and future trends," *Biomicrofluidics,* vol. 6, no. 1, p. 011301, 2012.

[14] A. Peiris and B. Stein, "Capillary Action," UC Davis, 2015. [Online]. Available: http://chemwiki.ucdavis.edu/Physical_Chemistry/Physical_Properties_of_Matter/Bulk_Properties/Cohesive_And_Adhesive_Forces/Capillary_Action. [Accessed 30 September 2015].

[15] A. W. Martinez, S. T. Phillips, M. J. Butte and G. M. Whitesides, "Patterned Paper as a Platform for Inexpensive, Low Volume, Portable Bioassays," *Angewandte Chemie International Edition English,* vol. 46, no. 8, pp. 1318-1320, 2007.

[16] E. Carrilho, A. W. Martinez and G. M. Whitesides, "Understanding Wax Printing: A Simple Micropatterning Process for Paper-Based Microfluidics," *Analytical Chemistry,* vol. 81, no. 16, pp. 7091-7095, 2009.

[17] G. G. Lewis, M. J. DiTucci, M. S. Baker and S. T. Phillips, "A High Throughput Method for Prototyping Three-Dimensional, Paper-Based Microfluidic Devices," *The Royal Society of Chemistry,* vol. 12, no. 15, pp. 2630-2633, 2012.

[18] GE Healthcare Life Sciences, "Grade 1 Chr Cellulose Chromatography Papers," GE, 2016. [Online]. Available: http://www.gelifesciences.com/webapp/wcs/stores/servlet/ProductDisplay?categoryId=104361&catalogId=10101&productId=14913&storeId=12751&langId=-1. [Accessed 28 February 2016].

[19] E. Carrilho, S. T. Phillips, S. J. Vella, A. W. Martinez and G. M. Whitesides, "Paper Microzone Plates," *Analytical Chemistry,* vol. 81, no. 15, pp. 5990-5998, 2009.

[20] Z. W. Zhong, Z. P. Wang and G. X. D. Huang, "Investigation of wax and paper materials for the fabrication of paper-based microfluidic devices," *Microsystem Technologies,* vol. 18, no. 5, pp. 649-659, 2012.

[21] IVD Technology, "IVD Technology Buyers Guide," UBM Canon, Plymouth, MN, 2012.

[22] Millipore Corporation, "Rapid Lateral Flow Test Strips: Considerations for Product Development," Millipore Corporation, Billerica, MA, 2008.

[23] K. Grifantini, "Lab-on-a-Chip Made of Paper," MIT Technology Review, 14 May 2008. [Online]. Available: http://www.technologyreview.com/news/410126/lab-on-a-chip-made-of-paper/. [Accessed 30 September 2015].

[24] F. Mearns, "Microfluidic devices made from only paper, a pen and sunlight," Royal Society of Chemistry, 10 September 2008. [Online]. Available: http://www.rsc.org/Publishing/ChemTech/Volume/2008/11/flashmicrofluidics.asp. [Accessed 30 November 2015].

[25] Thermo Fisher Scientific, "AB-0796 Model (1)," 07 May 2002. [Online]. Available: https://tools.thermofisher.com/content/sfs/manuals/MAN0014517_96well_u_bottom_qr.pdf. [Accessed 1 March 2016].

[26] EdgeBio, "96-Well Treated Microplates: U- Bottom," EdgeBio, 2016. [Online]. Available: https://www.edgebio.com/products/96-well-treated-microplates-u-bottom-50063. [Accessed 22 April 2016].

[27] Gilson, Inc., "PIPETMAN Neo® Multichannel," Gilson, Inc., 2016. [Online]. Available: http://www.gilson.com/Pipette/Products/47.260/Default.aspx#.VxrDlTArLIU. [Accessed 22 April 2016].

[28] A. W. Martinez, S. T. Phillips and G. M. Whitesides, "Three-dimensional microfluidic devices fabricated in layered paper and tape," *Proceedings of the National Academy of Sciences,* vol. 105, no. 50, p. 19606–19611, 2008.

[29] Cayman Chemical, "Glucose Colorimetric Assay Kit," Cayman Chemical, 2015. [Online]. Available: https://www.caymanchem.com/app/template/Product.vm/catalog/10009582. [Accessed 15 November 2015].

[30] Biomedica, "Veterinary," Biomedica, 2016. [Online]. Available: http://www.bmgrp.at/abteilungen/veterinaer-und-lebensmitteldiagnostik/veterinary/. [Accessed 1 April 2016].

[31] School of Veterinary Medicine, "Veterinary Diagnostic Services," University of Glasgow, [Online]. Available: http://www.gla.ac.uk/schools/vet/cad/. [Accessed 1 April 2016].

[32] T. J. Whitbread, "Urinalysis," The Merck Veterinary Manual, June 2015. [Online]. Available: http://www.merckvetmanual.com/mvm/clinical_pathology_and_procedures/diagnostic_procedures_for_the_private_practice_laboratory/urinalysis.html. [Accessed 30 September 2015].

[33] Moredun Research Institute, United Kingdom, "Disease Summaries," Moredun Research Institute, United Kingdom, 2015. [Online]. Available: http://www.moredun.org.uk/research/practical-animal-health-information/disease-summaries. [Accessed 30 September 2015].

[34] Animal & Plant Health Agency, "Livestock and Wildlife Disease Diagnosis at APHA: Guidance on sample and test selection," October 2014. [Online]. Available: http://ahvla.defra.gov.uk/documents/surveillance/sub-handbook.pdf. [Accessed 30 September 2015].

[35] World Health Organisation, "Simple / Rapid tests," World Health Organisation, 2016. [Online]. Available:

http://www.who.int/diagnostics_laboratory/faq/simple_rapid_tests/en/. [Accessed 20 March 2016].

[36] IDEXX Laboratories, Inc. , "Foot-and-Mouth Disease (FMD) Test Kit," IDEXX Laboratories, Inc. , 2016. [Online]. Available: http://www.idexx.co.uk/livestock-poultry/ruminant/foot-and-mouth.html. [Accessed 29 March 2016].

[37] Voigt Global Distribution, "Whatman Filter Paper," Voigt Global Distribution, 2016. [Online]. Available: http://www.vgdusa.com/whatman-filter-papers.htm. [Accessed 28 February 2016].

[38] Bristol Design Forge, "Laser Machine Hire Bristol," Bristol Design Forge, 2016. [Online]. Available: http://bristoldesignforge.co.uk/laser-machine-hire-bristol. [Accessed 21 April 2016].

[39] 3M, "Adhesive Transfer Tapes with Adhesive 300LSE," April 2013. [Online]. Available: http://solutions.3m.com/wps/portal/3M/en_US/Adhesives/Tapes/Products/~/3M-Adhesive-Transfer-Tape-9471FL-Clear-2-mil-Custom-Sizes-Available?N=8140106+4294864379&rt=rud. [Accessed 10 March 2016].

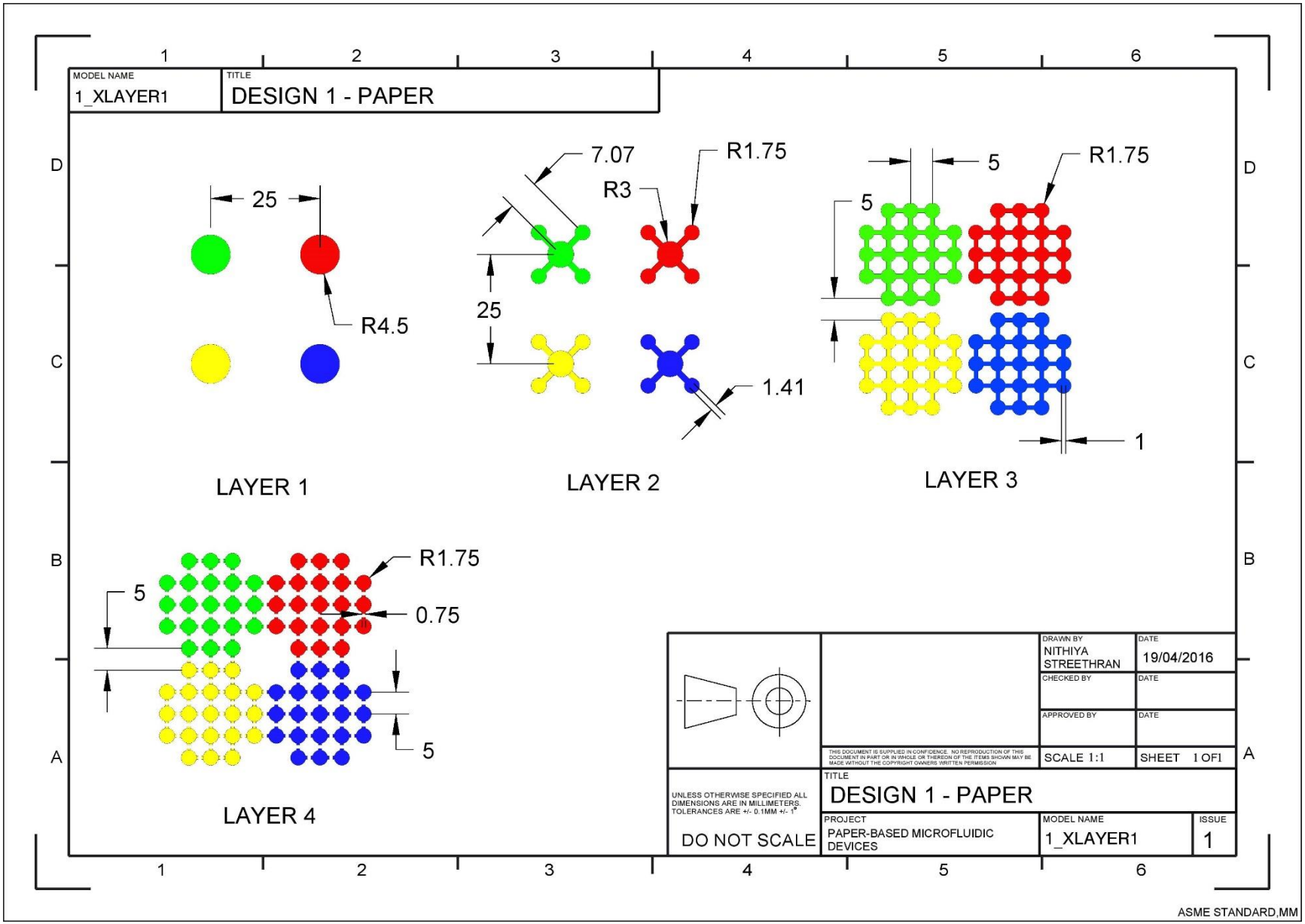
[40] Staples, "Staples Laminating Pouches A4," Staples, 2016. [Online]. Available: http://www.staples.co.uk/laminating-pouches/cbs/390355.html?promoCode=200200556&Effort_Code=WW&Find_Number=390355. [Accessed 28 February 2016].

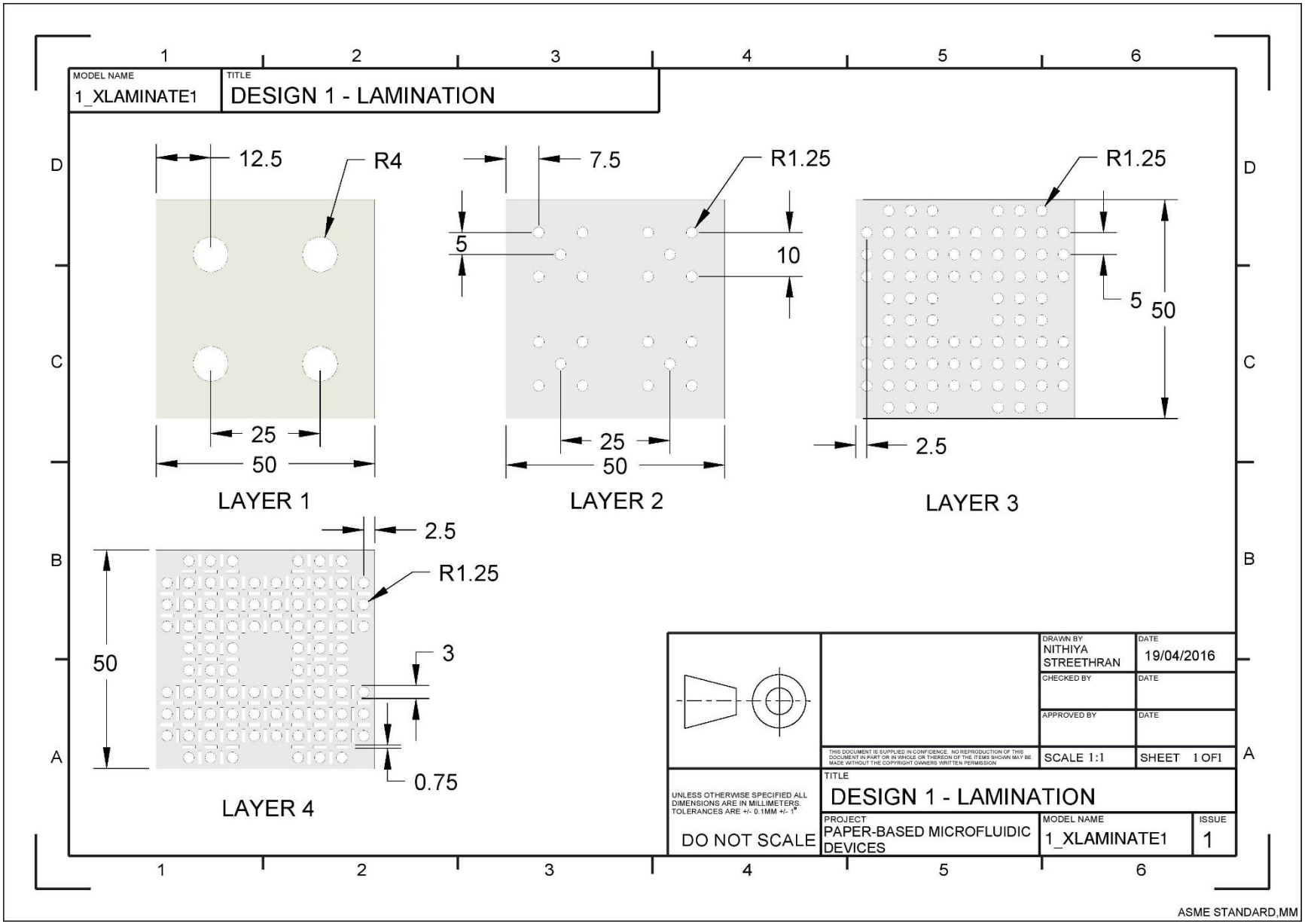
[41] Viking Tapes, "3M 9471 (300LSE Adhesive) Laminating Adhesive Rolls," Viking Industrial, 2016. [Online]. Available: http://www.vikingtapes.co.uk/p-556-3m-9471-300lse-adhesive-laminating-adhesive-rolls-305mm-x-55m.aspx#.VyMz8jArLIV. [Accessed 30 March 2016].

[42] M. Diez-Silva, M. Dao, J. Han, C.-T. Lim and S. Suresh, "Shape and Biomechanical Characteristics of Human Red Blood Cells in Health and Disease," *MRS Bulletin,* vol. 35, no. 5, pp. 382-388, 2010.

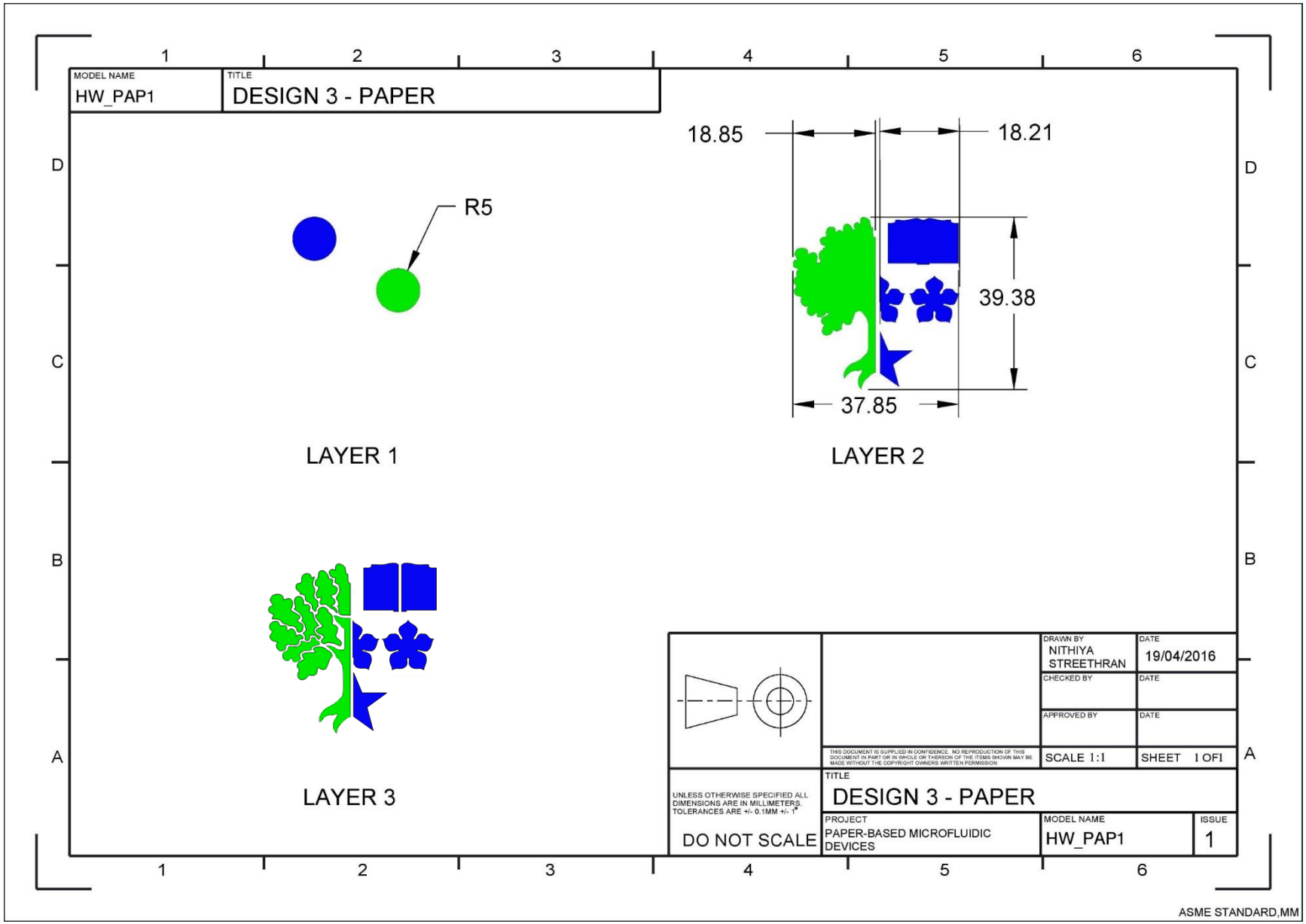
# Appendix A: Engineering Drawings of Designs

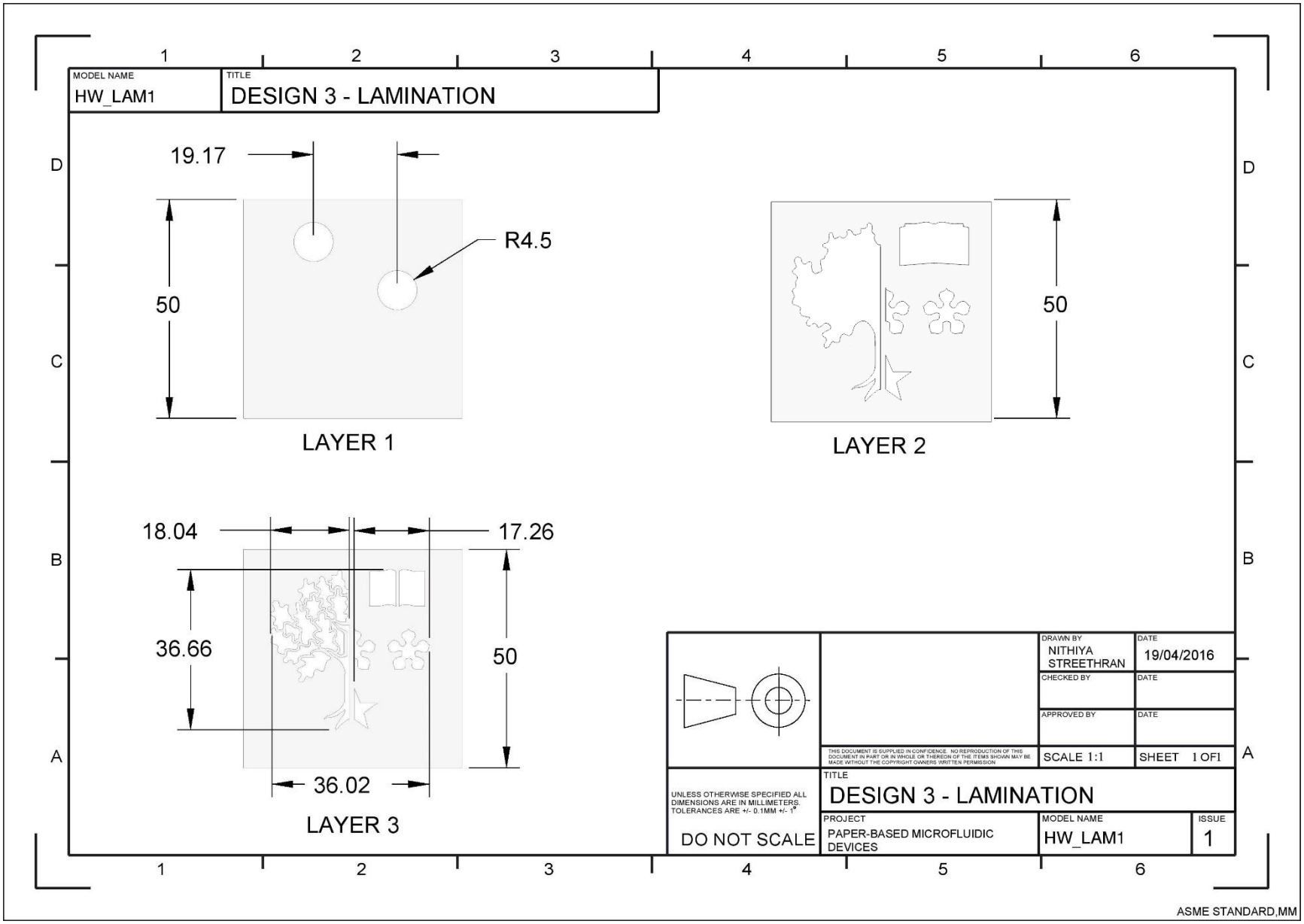
## Appendix A.i: Design 1



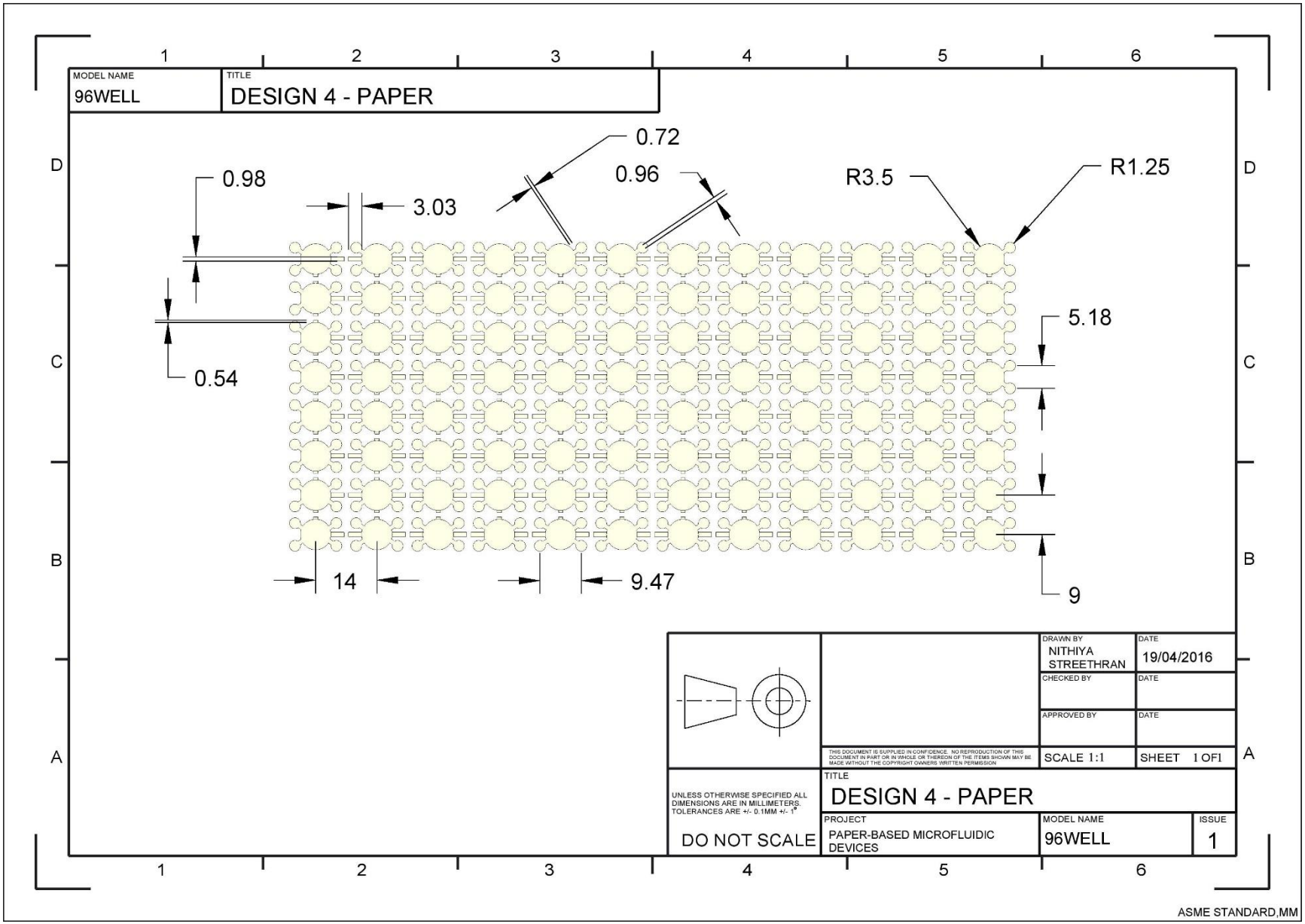

MODEL NAME
1_XLAMINATE1
TITLE
DESIGN 1 - LAMINATION
12.5
R4
25
50
LAYER 1
7.5
R1.25
5
10
25
50
LAYER 2
R1.25
5
50
2.5
LAYER 3
2.5
R1.25
50
3
0.75
LAYER 4
DRAWN BY
NITHIYA STREETHRAN
DATE
19/04/2016
CHECKED BY
DATE
APPROVED BY
DATE
SCALE 1:1
SHEET 1 OF1
UNLESS OTHERWISE SPECIFIED ALL DIMENSIONS ARE IN MILLIMETERS. TOLERANCES ARE +/- 0.1MM +/- 1°
DO NOT SCALE
TITLE
DESIGN 1 - LAMINATION
PROJECT
PAPER-BASED MICROFLUIDIC DEVICES
MODEL NAME
1_XLAMINATE1
ISSUE
1
ASME STANDARD,MM

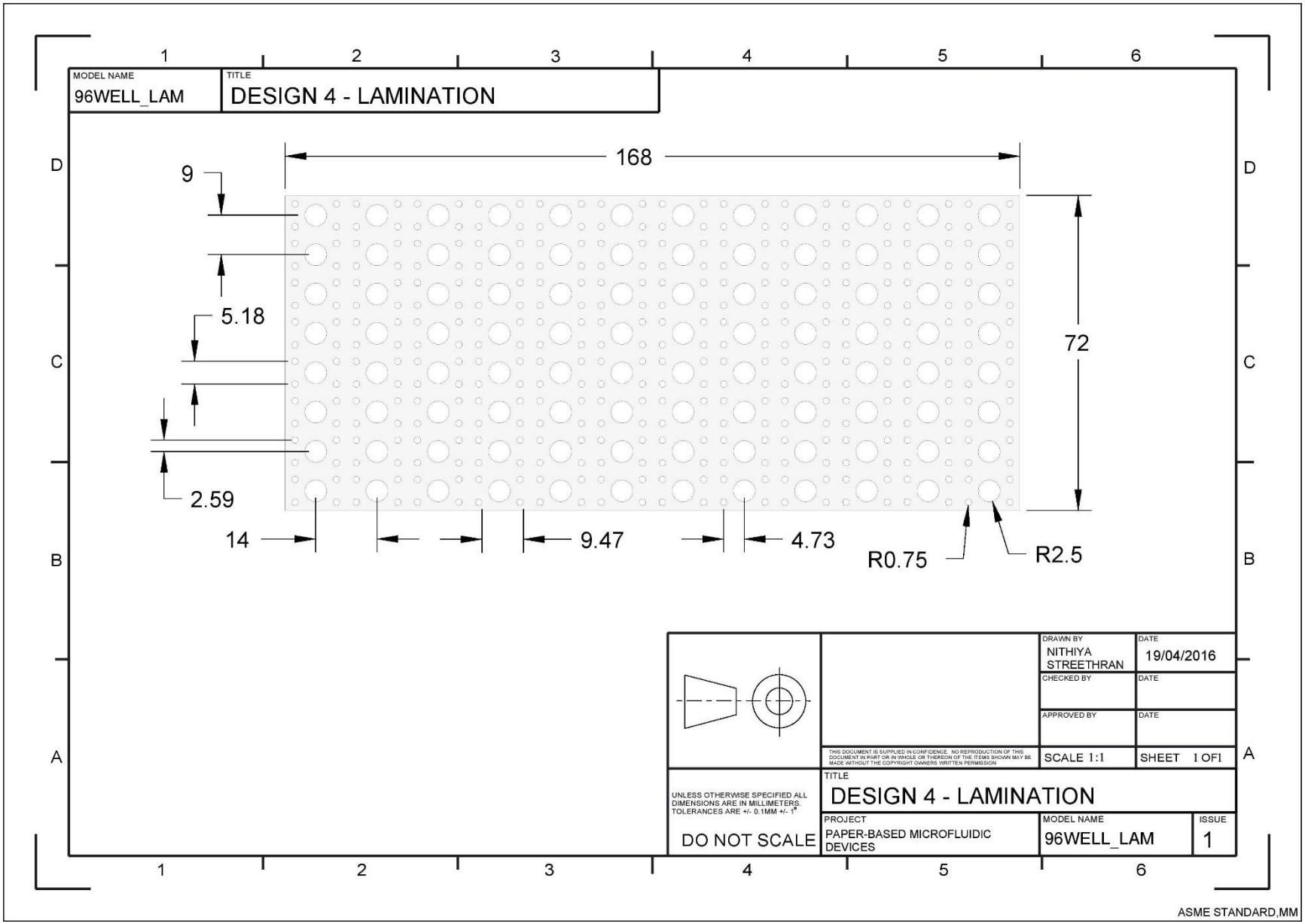
**Appendix A.ii: Design 2**




MODEL NAME
HW_LAM1
TITLE
DESIGN 3 - LAMINATION
19.17
R4.5
50
LAYER 1
50
LAYER 2
18.04
17.26
36.66
50
36.02
LAYER 3
DRAWN BY
NITHIYA STREETHRAN
DATE
19/04/2016
CHECKED BY
DATE
APPROVED BY
DATE
THIS DOCUMENT IS SUPPLIED IN CONFIDENCE. NO REPRODUCTION OF THIS DOCUMENT IN PART OR IN WHOLE OR THEREON OF THE ITEMS SHOWN MAY BE MADE WITHOUT THE COPYRIGHT OWNERS WRITTEN PERMISSION
SCALE 1:1
SHEET 1 OF1
UNLESS OTHERWISE SPECIFIED ALL DIMENSIONS ARE IN MILLIMETERS. TOLERANCES ARE +/- 0.1MM +/- 1°
DO NOT SCALE
TITLE
DESIGN 3 - LAMINATION
PROJECT
PAPER-BASED MICROFLUIDIC DEVICES
MODEL NAME
HW_LAM1
ISSUE
1
ASME STANDARD,MM

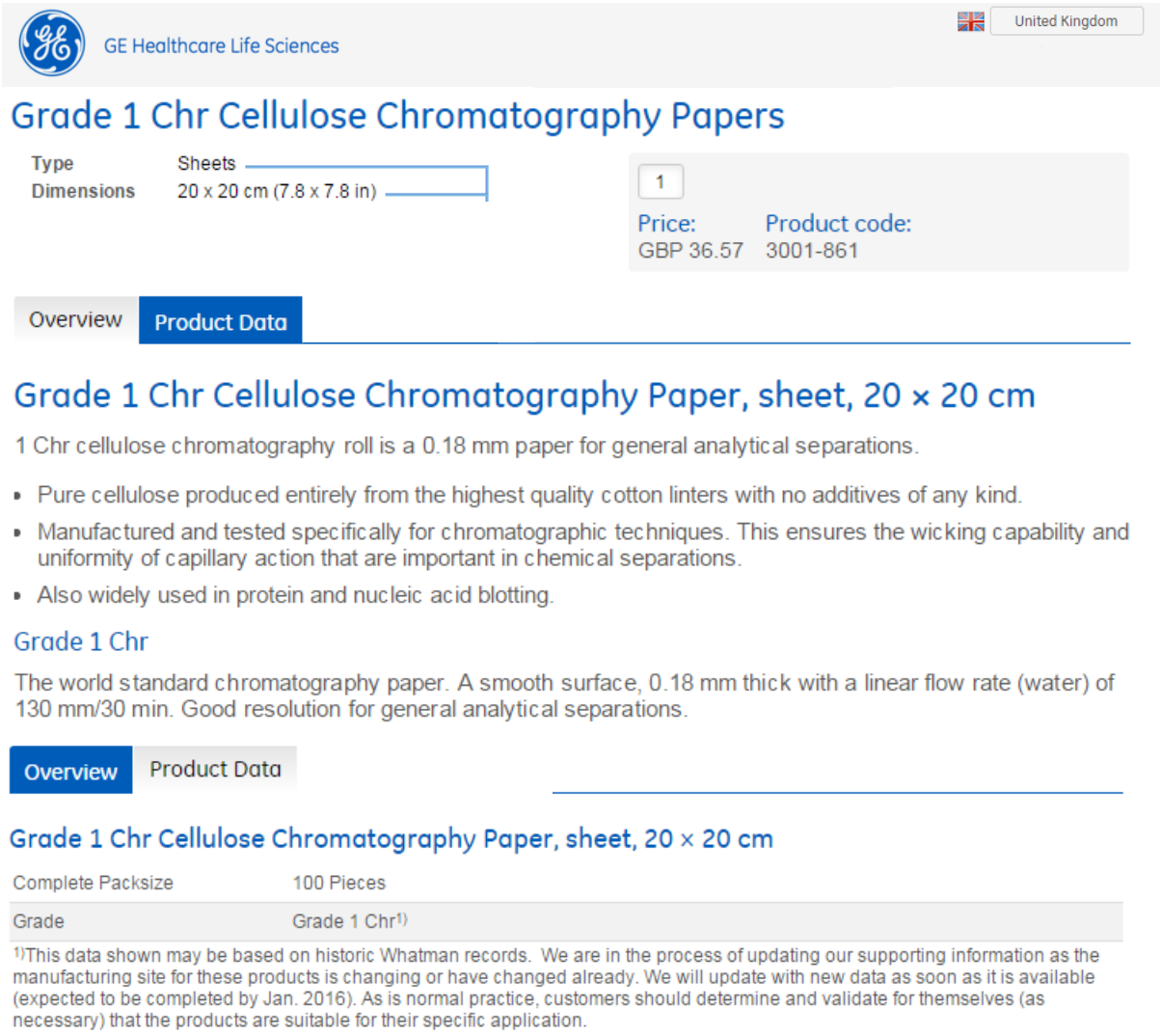
**Appendix A.iii: Design 3**




MODEL NAME
96WELL_LAM
TITLE
DESIGN 4 - LAMINATION
168
9
5.18
2.59
72
14
9.47
4.73
R0.75
R2.5
DRAWN BY
NITHIYA STREETHRAN
DATE
19/04/2016
CHECKED BY
DATE
APPROVED BY
DATE
THIS DOCUMENT IS SUPPLIED IN CONFIDENCE. NO REPRODUCTION OF THIS DOCUMENT IN PART OR IN WHOLE OR THEREON OF THE ITEMS SHOWN MAY BE MADE WITHOUT THE COPYRIGHT OWNERS WRITTEN PERMISSION
SCALE 1:1
SHEET 1 OF1
UNLESS OTHERWISE SPECIFIED ALL DIMENSIONS ARE IN MILLIMETERS. TOLERANCES ARE +/- 0.1MM +/- 1°
DO NOT SCALE
TITLE
DESIGN 4 - LAMINATION
PROJECT
PAPER-BASED MICROFLUIDIC DEVICES
MODEL NAME
96WELL_LAM
ISSUE
1
ASME STANDARD,MM

# Appendix B: Data Sheets

## Appendix B.i: Chromatography Paper [1]



GE Healthcare Life Sciences

United Kingdom

### Grade 1 Chr Cellulose Chromatography Papers

| | |
|---|---|
| Type | Sheets |
| Dimensions | 20 x 20 cm (7.8 x 7.8 in) |

1

Price: GBP 36.57

Product code: 3001-861

Overview | Product Data

### Grade 1 Chr Cellulose Chromatography Paper, sheet, 20 × 20 cm

1 Chr cellulose chromatography roll is a 0.18 mm paper for general analytical separations.

- Pure cellulose produced entirely from the highest quality cotton linters with no additives of any kind.
- Manufactured and tested specifically for chromatographic techniques. This ensures the wicking capability and uniformity of capillary action that are important in chemical separations.
- Also widely used in protein and nucleic acid blotting.

#### Grade 1 Chr

The world standard chromatography paper. A smooth surface, 0.18 mm thick with a linear flow rate (water) of 130 mm/30 min. Good resolution for general analytical separations.

Overview | Product Data

### Grade 1 Chr Cellulose Chromatography Paper, sheet, 20 × 20 cm

| | |
|---|---|
| Complete Packsize | 100 Pieces |
| Grade | Grade 1 Chr[1)] |

[1)]This data shown may be based on historic Whatman records. We are in the process of updating our supporting information as the manufacturing site for these products is changing or have changed already. We will update with new data as soon as it is available (expected to be completed by Jan. 2016). As is normal practice, customers should determine and validate for themselves (as necessary) that the products are suitable for their specific application.

[1] GE Healthcare Life Sciences, "Grade 1 Chr Cellulose Chromatography Papers," GE, [Online]. Available: http://www.gelifesciences.com/webapp/wcs/stores/servlet/ProductDisplay?categoryId=104361&catalogId=10101&productId=14913&storeId=12751&langId=-1. [Accessed 28 February 2016].

## Appendix B.ii: Lamination Pouch [2]


[2] Staples, "Staples Laminating Pouches A4," Staples, [Online]. Available: http://www.staples.co.uk/laminating-pouches/cbs/390355.html. [Accessed 28 February 2016].

## Appendix B.iii: Adhesive Transfer Tape [3]

3M

# Adhesive Transfer Tapes with Adhesive 300LSE

8132LE • 8153LE • 9453LE • 9471LE • 9472LE
9653LE • 9671LE • 9672LE

**Technical Data** **April, 2013**

**Product Description**

3M™ Adhesive Transfer Tapes with 3M™ Low Surface Energy Acrylic Adhesive 300LSE provides high bond strength to most surfaces, including many low surface energy plastics such as polypropylene and powder coated paints. The acrylic adhesive also provides excellent adhesion to surfaces contaminated lightly with oil typically used with machine parts.

**Product Construction**

| Product Number | Adhesive: (Solvent Free) | Liner: |
|---|---|---|
| 3M™ Adhesive Transfer Tape 8132LE | 2.3 mils (58 microns) 3M High Strength Acrylic Adhesive 300LSE | 4.0 mils (100 microns) 58# Polycoated kraft 6.2 mils (157 microns) 83# Polycoated kraft |
| 3M™ Adhesive Transfer Tape 8153LE | 3.5 mils (88 microns) 3M High Strength Acrylic Adhesive 300LSE | 4.0 mils (100 microns) 58# Polycoated kraft 6.2 mils (157 microns) 83# Polycoated kraft |
| 3M™ Adhesive Transfer Tape 9453LE | 3.6 mils (91 microns) 3M High Strength Acrylic Adhesive 300LSE | 4.2 mils (107 microns) 58# Polycoated kraft |
| 3M™ Adhesive Transfer Tape 9471LE | 2.3 mils (58 microns) 3M High Strength Acrylic Adhesive 300LSE | 4.2 mils (107 microns) 58# Polycoated kraft |
| 3M™ Adhesive Transfer Tape 9472LE | 5.2 mils (132 microns) 3M High Strength Acrylic Adhesive 300LSE | 4.2 mils (107 microns) 58# Polycoated kraft |
| 3M™ Adhesive Transfer Tape 9653LE | 3.5 mils (88 microns) 3M High Strength Acrylic Adhesive 300LSE | 6.2 mils (157 microns) 83# Polycoated kraft |
| 3M™ Adhesive Transfer Tape 9671LE | 2.3 mils (58 microns) 3M High Strength Acrylic Adhesive 300LSE | 6.2 mils (157 microns) 83# Polycoated kraft |
| 3M™ Adhesive Transfer Tape 9672LE | 5.0 mils (127 microns) 3M High Strength Acrylic Adhesive 300LSE | 6.2 mils (157 microns) 83# Polycoated kraft |

[3] 3M, "Adhesive Transfer Tapes with Adhesive 300LSE," [Online]. Available: http://solutions.3m.com/wps/portal/3M/en_US/Adhesives/Tapes/Products/~/3M-Adhesive-Transfer-Tape-9471FL-Clear-2-mil-Custom-Sizes-Available?N=8140106+4294864379&rt=rud. [Accessed 10 March 2016].

# 3M™ Adhesive Transfer Tapes with Adhesive 300LSE

8132LE • 8153LE • 9453LE • 9471LE • 9472LE • 9653LE • 9671LE • 9672LE • 9698LE

**Typical Physical Properties and Performance Characteristics**

**Typical Adhesion Properties**

**Note: The following technical information and data should be considered representative or typical only and should not be used for specification purposes.**

Peel Adhesion - ounces/inch (Newtons/100 mm) ASTM D3330, modified: 90° peel, 2 mil aluminum backing.

**Typical Adhesion Chart**

| | 3M™ Adhesive Transfer Tape | 15 Minute Room Temperature Oz./In. | 15 Minute Room Temperature N/100 mm | 72 Hour Room Temperature Oz./In. | 72 Hour Room Temperature N/100 mm |
|---|---|---|---|---|---|
| Stainless Steel | 8132LE | 71 | 78 | 75 | 82 |
| | 8153LE | 90 | 98 | 100 | 109 |
| ABS | 8132LE | 70 | 77 | 79 | 86 |
| | 8153LE | 80 | 88 | 113 | 124 |
| Polypropylene | 8132LE | 69 | 75 | 74 | 81 |
| | 8153LE | 89 | 97 | 103 | 113 |
| Stainless Steel | 9453LE | 90 | 98 | 100 | 109 |
| | 9471LE | 71 | 78 | 75 | 82 |
| | 9472LE | 109 | 119 | 140 | 153 |
| ABS | 9453LE | 80 | 88 | 113 | 124 |
| | 9471LE | 70 | 77 | 79 | 86 |
| | 9472LE | 102 | 112 | 128 | 140 |
| Polypropylene | 9453LE | 89 | 97 | 103 | 113 |
| | 9471LE | 69 | 75 | 74 | 81 |
| | 9472LE | 115 | 126 | 136 | 149 |
| Stainless Steel | 9653LE | 90 | 98 | 100 | 109 |
| | 9671LE | 71 | 78 | 75 | 82 |
| | 9672LE | 109 | 119 | 140 | 153 |
| ABS | 9653LE | 80 | 88 | 113 | 124 |
| | 9671LE | 70 | 77 | 79 | 86 |
| | 9672LE | 102 | 112 | 128 | 140 |
| Polypropylene | 9653LE | 89 | 97 | 103 | 113 |
| | 9671LE | 69 | 75 | 74 | 81 |
| | 9672LE | 115 | 126 | 136 | 149 |

# 3M™ Adhesive Transfer Tapes with Adhesive 300LSE

8132LE • 8153LE • 9453LE • 9471LE • 9472LE • 9653LE • 9671LE • 9672LE

**Typical Physical Properties and Performance Characteristics *(continued)***

**Typical Adhesion Properties**

**Note: The following technical information and data should be considered representative or typical only and should not be used for specification purposes.**

The properties defined are based on the attachment of impervious faceplate materials (such as aluminum) to a stainless steel test surface.

**Bond Build-up:** The bond strength of 3M™ Adhesive 300LSE increased as a function of time and temperature, and has very high initial adhesion.

**Humidity Resistance:** High humidity has a minimal effect on adhesive performance. No significant reduction in bond strength is observed after exposure for 7 days at 90°F (32°C) and 90% relative humidity.

**U.V. sistance:** When properly applied, nameplates and decorative trim parts are not adversely affected by exposure.

**Water Resistance:** Immersion in water has no appreciable effect on the bond strength. After 100 hours at room temperature, the high bond strength is maintained.

**Temperature Cycling Resistance:** High bond strength is maintained after cycling four times through:

4 hours at 158°F (70°C)
4 hours at -20°F (-29°C)
4 hours at 73°F (22°C)

**Chemical Resistance:** When properly applied, nameplates and decorative trim parts will hold securely after exposure to numerous chemicals including oil, mild acids and alkalis.

**Temperature Resistance:** 3M™ Adhesive 300LSE is usable for short periods (minutes, hours) at temperatures up to 300°F (148°C) and for intermittent longer periods of time (days, weeks) up to 200°F (93°C).

**Lower Service Temperature:** -40°F (-40°C).

**Available Sizes**

Width and Length (subject to minimum order requirements):

| | **3M™ Adhesive Transfer Tapes 8132LE, 8153LE*** | **3M™ Adhesive Transfer Tapes 9453LE, 9471LE, 9472LE, 9653LE, 9671LE, 9672LE** |
|---|---|---|
| Standard Sheet Size: | 24 in. x 36 in. | – |
| Limitations: | Maximum 360 yards | 1/2 in. to 63/64 in.: Maximum 180 yards<br>1 in. to 54 in.: Maximum 360 yards |
| Minimum Slit Width: | 12 in. | 1/2 in. |
| Maximum Slit Width: | 48 in. | 54 in. |
| Normal Slitting Tolerance: | ± 1/32 in. | ± 1/32 in. |
| Core | 6.0 in. | 3.0 in. |

*Custom sheets are available.

# 3M™ Adhesive Transfer Tapes with Adhesive 300LSE

8132LE • 8153LE • 9453LE • 9471LE • 9472LE • 9653LE • 9671LE • 9672LE • 9698LE

**Features**

- 3M™ Adhesive 300LSE is a hi-strength acrylic adhesive that provides a very high bond strength to most surfaces.
- Excellent bond to low surface energy plastics such as, polypropylene and powder coatings.
- Excellent adhesion to lightly oiled surfaces typical of machine parts.
- Thickness range of 2.0 mils, 3.5 mils, 5.0 mils and 8.5 mils for use on smooth, or rough surfaces.
- Extremely smooth adhesive for excellent graphics appearance.
- Double linered for selective die-cutting.
- Polycoated kraft liner for die-cutting end tabs and waste removed nameplates on a common carrier.
- 3M™ Adhesive Transfer Tapes 8132LE and 8153LE are double linered for selective die-cutting.

**Application Techniques**

For maximum bond strength, the surface should be thoroughly cleaned and dried. Typical cleaning solvents are heptane or isopropyl alcohol. Carefully read and follow manufacturer's precautions and directions for use when using cleaning solvents.

Bond strength can also be improved with firm application pressure and moderate heat, from 100°F (38°C) to 130°F (54°C), causing the adhesive to develop intimate contact with the bonding surface.

Ideal tape application temperature range is 70°F to 100°F (21°C to 38°C). Initial tape application to surfaces at temperatures below 50°F (10°C) is not recommended for most pressure-sensitive adhesives because the adhesive becomes too firm to adhere readily. However, once properly applied, low temperature holding is generally satisfactory.

**General Information**

- Plastic nameplates or graphic overlays for use on low surface energy plastics.
- Waste removed nameplates on a common sheet for ease of application.
- Attaching membrane switch assemblies to powder coated surfaces and low surface energy plastics.
- Graphic overlays with end tabs for easy liner removal.
- Graphic application to surfaces such as wood, fabric, plastic, where very high bond strength is required.
- Attaching identification material to lightly oily surfaces typical of machine parts.

**Application Ideas**

**Processing:**

**Slitting and die-cutting:** This adhesive is very aggressive and may be difficult to convert depending on your application requirements. Chilling the adhesive between 35°F and 50°F will improve the processability. In addition, dies can be lubricated with Laminoleum evaporative stamping oil, which is available from Metal Lubricants Company (708-333-8900), or with Lubri-Blade 907 from Ceramic Technologies Inc. (800-258-8495). You may also refer to our Technical Bulletin on 3M™ Adhesive 300LSE converting. (70-0707-6205-2)

**Roll Laminating:** A combination of metal and rubber rollers with moderate pressure is recommended.

**Note:** Please refer to the Technical Bulletin on slitting. (70-0709-3905-6)

# 3M™ Adhesive Transfer Tapes with Adhesive 300LSE

8132LE • 8153LE • 9453LE • 9471LE • 9472LE • 9653LE • 9671LE • 9672LE

| | |
|---|---|
| **Storage** | Store at room temperature conditions of 70°F (21°C) and 50% relative humidity. |
| **Shelf Life** | If stored properly, product retains its performance and properties for 18 months from date of shipment. |
| **Recognition/ Certification** | **TSCA:** These products are defined as articles under the Toxic Substances Control Act and therefore, are exempt from inventory listing requirements.<br>**MSDS:** These products are not subject to the MSDS requirements of the Occupational Safety and Health Administration's Hazard Communication Standard, 29 C.F.R. 1910.1200(b)(6)(v). When used under reasonable conditions or in accordance with the 3M directions for use, the products should not present a health and safety hazard. However, use or processing of the products in a manner not in accordance with the directions for use may affect their performance and present potential health and safety hazards.<br>**Note:** One of 3M's core values is to respect our social and physical environment. 3M is committed to comply with ever-changing, global, regulatory and consumer environmental, health, and safety (EHS) requirements. As a service to our customers, 3M is providing information on the regulatory status of many 3M products. Further regulation information including that for OSHA, USCPSI, FDA, California Proposition 65, READY and RoHS, can be found at 3M.com/regs. |
| **Technical Information** | The technical information, recommendations and other statements contained in this document are based upon tests or experience that 3M believes are reliable, but the accuracy or completeness of such information is not guaranteed. |
| **Product Use** | Many factors beyond 3M's control and uniquely within user's knowledge and control can affect the use and performance of a 3M product in a particular application. Given the variety of factors that can affect the use and performance of a 3M product, user is solely responsible for evaluating the 3M product and determining whether it is fit for a particular purpose and suitable for user's method of application. |
| **Warranty, Limited Remedy, and Disclaimer** | Unless an additional warranty is specifically stated on the applicable 3M product packaging or product literature, 3M warrants that each 3M product meets the applicable 3M product specification at the time 3M ships the product. 3M MAKES NO OTHER WARRANTIES OR CONDITIONS, EXPRESS OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, ANY IMPLIED WARRANTY OR CONDITION OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR ANY IMPLIED WARRANTY OR CONDITION ARISING OUT OF A COURSE OF DEALING, CUSTOM OR USAGE OF TRADE. If the 3M product does not conform to this warranty, then the sole and exclusive remedy is, at 3M's option, replacement of the 3M product or refund of the purchase price. |
| **Limitation of Liability** | Except where prohibited by law, 3M will not be liable for any loss or damage arising from the 3M product, whether direct, indirect, special, incidental or consequential, regardless of the legal theory asserted, including warranty, contract, negligence or strict liability. |

ISO 9001

This Industrial Adhesives and Tapes Division product was manufactured under a 3M quality system registered to ISO 9001 standards.

3M

**Industrial Adhesives and Tapes Division**
**Converter Markets**
3M Center, Building 225-3S-06
St. Paul, MN 55144-1000
800-223-7427 • 651-778-4244 (fax)
www.3M.com/converter

*Recycled Paper*
*40% pre-consumer*
*10% post-consumer*

## Appendix B.iv: Food Colouring [4]

Sainsbury's

Sainsbury's Blue Food Colouring 38ml

**£1.00/unit** £0.26/10ml

Item code: 7609596

Information

**Description**

Blue Food Colouring

**Ingredients**

Water, Vegetable Glycerine, Plant concentrate (Spirulina) Acidity Regulator: Citric Acid.

**Health**

No artificial colours or flavours

**Preparation and Safety**

**Cooking instructions: other**

Not suitable for baking.

**Country of Origin**

Produced in United Kingdom

**Size**

38ml

**Storage**

Shake well before use. Store in a cool dry place away from strong light. Once opened keep refrigerated.

**Packaging**

RPET / PET bottle

Aluminium cap

Aluminium cap

Paper laminate label

Paper laminate label

**Manufacturer**

We are happy to replace this item if it is not satisfactory

Sainsbury's Supermarkets Ltd.

33 Holborn, London EC1N 2HT

Customer services 0800 636262

**Important Information**

The above details have been prepared to help you select suitable products. Products and their ingredients are liable to change.

**You should always read the label before consuming or using the product and never rely solely on the information presented here.**

If you require specific advice on any Sainsbury's branded product, please contact our Customer Careline on 0800 636262. For all other products, please contact the manufacturer.

This information is supplied for your personal use only. It may not be reproduced in any way without the prior consent of Sainsbury's Supermarkets Ltd and due acknowledgement.

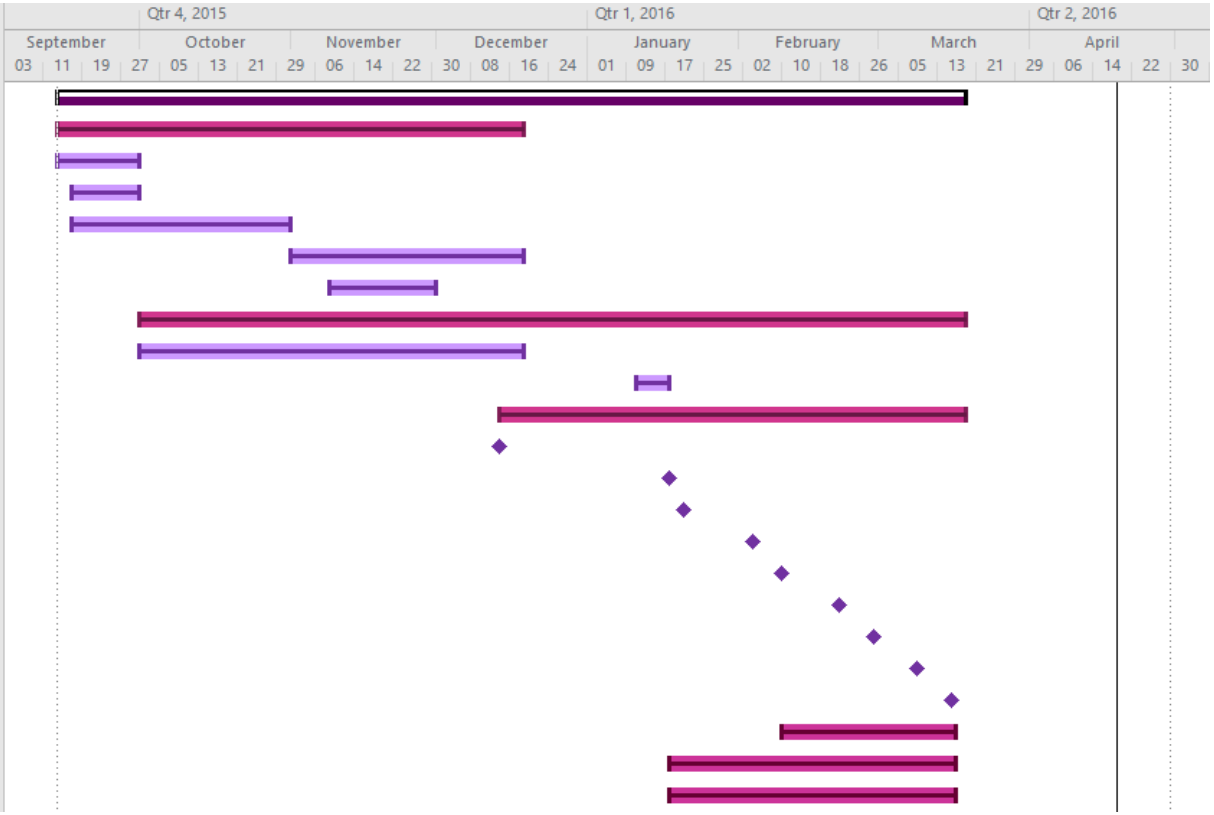
7609596

[4] Sainsbury's, "Sainsbury's Blue Food Colouring 38ml," Sainsbury's, [Online]. Available: http://www.sainsburys.co.uk/shop/gb/groceries/sainsburys-blue-food-colouring-38ml. [Accessed 18 March 2016].

# Appendix C: Project Management

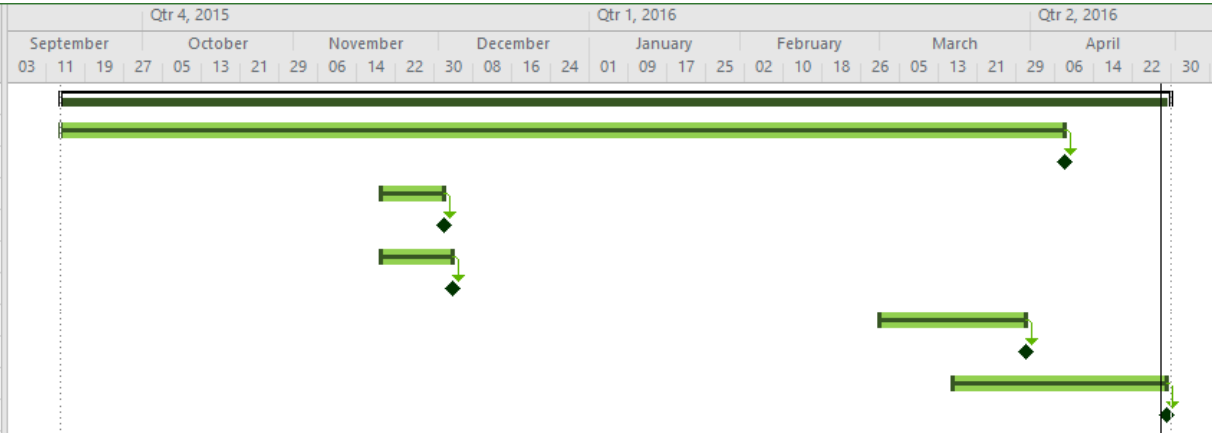
## Appendix C.i: Project Work

| Task Name | Start | Finish |
|---|---|---|
| **Project Work** | **Mon 14/09/15** | **Fri 18/03/16** |
| **1 Research** | Mon 14/09/15 | Fri 18/12/15 |
| 1.1 Initial Research | Mon 14/09/15 | Wed 30/09/15 |
| 1.2 Fabrication Methods | Thu 17/09/15 | Wed 30/09/15 |
| 1.3 Existing Technologies | Thu 17/09/15 | Sat 31/10/15 |
| 1.4 Further Research | Sun 01/11/15 | Fri 18/12/15 |
| 1.5 Literature Review | Mon 09/11/15 | Mon 30/11/15 |
| **2 Design** | Thu 01/10/15 | Fri 18/03/16 |
| 2.1 Design Concepts | Thu 01/10/15 | Fri 18/12/15 |
| 2.2 Final Designs | Mon 11/01/16 | Sun 17/01/16 |
| **3 Fabrication** | Mon 14/12/15 | Fri 18/03/16 |
| 3.1 Training | Mon 14/12/15 | Mon 14/12/15 |
| 3.2 Fabrication 1 | Mon 18/01/16 | Mon 18/01/16 |
| 3.3 Fabrication 2 | Thu 21/01/16 | Thu 21/01/16 |
| 3.4 Fabrication 3 | Thu 04/02/16 | Thu 04/02/16 |
| 3.5 Fabrication 4 | Wed 10/02/16 | Wed 10/02/16 |
| 3.6 Fabrication 5 | Mon 22/02/16 | Mon 22/02/16 |
| 3.7 Fabrication 6 | Mon 29/02/16 | Mon 29/02/16 |
| 3.8 Fabrication 7 | Wed 09/03/16 | Wed 09/03/16 |
| 3.9 Fabrication 8 | Wed 16/03/16 | Wed 16/03/16 |
| **4 Testing** | Wed 10/02/16 | Wed 16/03/16 |
| **5 Analysis of Results** | Mon 18/01/16 | Wed 16/03/16 |
| **6 Optimisation** | Mon 18/01/16 | Wed 16/03/16 |



## Appendix C.ii: Assessment

| Task Name | Start | Finish |
|---|---|---|
| **Assessment** | **Mon 14/09/15** | **Fri 29/04/16** |
| **1 Project Log** | Mon 14/09/15 | Thu 07/04/16 |
| Project Log due | Fri 08/04/16 | Fri 08/04/16 |
| **2 Interim Presentation** | Thu 19/11/15 | Tue 01/12/15 |
| Interim Presentation day | Wed 02/12/15 | Wed 02/12/15 |
| **3 Progress Report** | Thu 19/11/15 | Thu 03/12/15 |
| Progress Report due | Fri 04/12/15 | Fri 04/12/15 |
| **4 Major Presentation** | Tue 01/03/16 | Wed 30/03/16 |
| Major Presentation day | Thu 31/03/16 | Thu 31/03/16 |
| **5 Journal Paper** | Wed 16/03/16 | Thu 28/04/16 |
| Journal Paper due | Fri 29/04/16 | Fri 29/04/16 |



## Appendix C.iii: Meetings and Updates with Supervisor

| Task Name | Start | Finish |
|---|---|---|
| **Meetings and Updates with Supervisor** | **Thu 17/09/15** | **Fri 29/04/16** |
| **1 Semester 1** | Thu 17/09/15 | Fri 04/12/15 |
| 1.1 Meeting 1 | Thu 17/09/15 | Thu 17/09/15 |
| 1.2 Weekly Report 1 | Thu 17/09/15 | Wed 23/09/15 |
| 1.3 Short Presentation 1 | Thu 24/09/15 | Tue 29/09/15 |
| 1.4 Meeting 2 | Wed 30/09/15 | Wed 30/09/15 |
| 1.5 Weekly Report 2 | Thu 01/10/15 | Wed 07/10/15 |
| 1.6 Short Presentation 2 | Thu 08/10/15 | Wed 14/10/15 |
| 1.7 Weekly Report 3 | Thu 15/10/15 | Wed 21/10/15 |
| 1.8 Short Presentation 3 | Thu 22/10/15 | Tue 27/10/15 |
| 1.9 Meeting 3 | Wed 28/10/15 | Wed 28/10/15 |
| 1.10 Short Presentation 4 | Thu 29/10/15 | Wed 04/11/15 |
| 1.11 Weekly Report 4 | Thu 05/11/15 | Wed 11/11/15 |
| 1.12 Short Presentation 5 | Thu 12/11/15 | Tue 17/11/15 |
| 1.13 Meeting 4 | Wed 18/11/15 | Wed 18/11/15 |
| 1.14 Mock Interim Presentation | Thu 19/11/15 | Tue 24/11/15 |
| 1.15 Meeting 5 | Wed 25/11/15 | Wed 25/11/15 |
| 1.16 Interim Presentation | Thu 19/11/15 | Tue 01/12/15 |
| 1.17 Interim Presentation day | Wed 02/12/15 | Wed 02/12/15 |
| 1.18 Progress Report | Thu 19/11/15 | Fri 04/12/15 |
| **2 Semester 2** | Fri 12/02/16 | Fri 29/04/16 |
| 2.1 Short Presentation 6 | Fri 12/02/16 | Thu 18/02/16 |
| 2.2 Meeting 6 | Fri 19/02/16 | Fri 19/02/16 |
| 2.3 Mock Presentation | Sat 20/02/16 | Tue 22/03/16 |
| 2.4 Meeting 7 | Tue 22/03/16 | Tue 22/03/16 |
| 2.4 Meeting 8 | Wed 23/03/16 | Wed 23/03/16 |
| 2.5 Major Presentation | Wed 16/03/16 | Wed 30/03/16 |
| 2.6 Major Presentation day | Thu 31/03/16 | Thu 31/03/16 |
| 2.7 Journal Paper | Thu 17/03/16 | Fri 29/04/16 |