# APP N17-3: Nanotechnology Design Stage 3

Engineering 1282.02H

Spring 2018

Y5 - Manotech
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D. Grzybowski MWF 12:40 p.m.
K. Kolotka

Date of Submission: 4/6/18

## I. Questions

a. What do you want your device to be able to diagnose? Potential impact of device?

The created NANOLYSER would be able to detect the presence of Celiac Disease in a patient given a single drop of their blood. Celiac Disease is an autoimmune disease that occurs when the body overreacts to ingested gluten. The body attacks the gluten and in the process also damages parts of the body, mainly the villi in the small intestine. This can cause symptoms of abdominal pain, vomiting, diarrhea, constipation, and many others. It affects nearly 1% of the population, which indicates its importance for an easy, quick detection method. Previously, celiac disease required an invasive and expensive intestinal tissue biopsy, or large-volume blood work with a long turnaround time. A simple lab-on-a-chip device would simultaneously reduce time of analysis, cost of detection, and blood required from the patient. An easy detection method is important to decrease the damage on the body from eating gluten.

b. What type of analyte will you assess (e.g., cell shape, cell surface proteins, DNA)?

The NANOLYSER will assess the presence of anti-tissue transglutaminase (anti-tTg), which is an antibody created by the body as a response to celiac disease.

c. How will the blood sample and reagents be loaded into the NANOLYSER?

The blood sample will be loaded into a micropipette and injected into the NANOLYSER through a hole that is the same size as the tip of the micropipette. The test solution will be loaded into the testing chamber prior to assembly of the chip, and the gluten solution will be loaded into the chamber adjacent to the testing chamber to allow for later mixing.

d. How will samples and reagents be moved around and mixed in the NANOLYSER?

The blood will be transported to the testing chamber via capillary action that is controlled by hydrophilic fluoropolymer micro-capillary film coatings. The gluten solution will be stored in a chamber adjacent to the testing chamber, and a mechanical, operable door will connect the two chambers so that the gluten solution can mix with the test solution at the user’s discretion.

e. Will you isolate the target analyte from the other blood components or analyze the whole blood mixture? Reasoning for this decision? Approach if isolating analyte?

The blood cells with be filtered from the sample, this is because the basic reagents present in the reaction chamber will destroy the cells and the red hemoglobin present in the cells will interfere with the color indicator and calorimetry. The blood will be mechanically filtered by a mesh so that the large blood cell separate from the rest of the blood serum containing the analyte.

f. What will be different processing steps (e.g.,cell separation, lysis, DNA labeling)? Will these be performed in the same location or in different chambers?

Once loaded into the chip the blood will first have the red blood cells mechanically separated from the blood serum. Several solutions will also need to be combined in steps. The tTG and sodium hypochlorite (one of the indicator reagents) will mix with the filtered blood first, then combined with the Sodium salicylate and sodium nitroferricyanide to form the indicator solution. Then the gluten sample will be introduced last to allow the NH3 production to start.

g. How will the NANOLYSER be read (e.g., fluorescence reader)?

The chip will be read through the color change in the NH3 indicator solution. Qualitative tests can be performed as no color change indicates no NH3 produced and the Anti-tTG is present. Or using a standardized concentration of the indicator colorimetry can be used to determine the quantitative amount of NH3 produced in the reaction.

h. Will the NANOLYSER be disposable or reusable?

The NANOLYSER is designed to be separable into two parts, so after the analysis has been performed, the two pieces of the device can be re-separated, sonicated, and re-loaded with solution. Thus, the chip is completely reusable.

i. What material(s) will your device be made of?

The device will be made from clear acrylic plastic; this is necessary since the color of the solution in the testing chamber must be visible at all times and be able to be analyzed using a colorimeter.

j. How will your device be fabricated?

The NANOLYSER will be milled in two parts using a CNC machine. The pull valve will be die cut from rubber, and placed in the bottom half. The blood filter will be purchased separately and placed in the bottom half. Every needed solution will then be placed in their given chamber, and the two parts of the chip will be attached together.

**II. Blood Processing Algorithm**

1. Blood deposited into entry well via micropipette.
2. Blood flows through filter with 5 uM pores, filtering out RBCs and allowing anti-tTG to pass through.
3. Anti-tTG in remaining blood fluid flows into chamber A, where a solution containing tTG/$Ca^{2+}$ and sodium hypochlorite (bleach) is preloaded.
4. After waiting 30 seconds, pull up on handle on top of chip to open valve, allowing contents of chamber B to flow into chamber A.
5. Aqueous sodium salicylate, aqueous sodium nitroferricyanide, and suspended wheat gliadin flow from chamber B into chamber A.
6. In a sample negative for anti-tTG, tTG catalyzes the conversion of wheat gliadin into products including ammonia ($NH_3$).
7. Excess ammonia reacts with hypochlorite ions and salicylate ions, catalyzed by nitroferricyanide, to produce the deeply blue colored indosalicylate. (The same reaction is used in ammonia test strips).
8. In a sample positive for anti-tTG, tTG's activity is inhibited by the binding of anti-tTG, preventing the conversion of wheat gliadin into ammonia.
9. In the absence of excess ammonia, the yellow color of unreacted nitroferricyanide remains.

**III. Results Analysis**

If a deep blue color is observed, it can be stated that the sample is negative for anti-tTG, and that the patient does not have Celiac Disease. This is because a deep blue color indicates a high concentration of ammonia, produced through a high activity of tTG, which indicates that anti-tTG is not present to inhibit tTG.

If a pale yellow color is observed it can be stated that the sample is positive for anti-tTG, and that the patient likely has Celiac Disease. This is because a pale yellow color indicates a low concentration of ammonia, showing high inhibition of tTG through the action of anti-tTG.

If a green color is observed, the test is inconclusive based on inspection alone. The NANOLYSER should be measured in a colorimeter to determine the absorbance at 650 nm, the wavelength absorbed by indosalicylate.

If this inconclusive reading was taken while the patient was on a gluten-containing diet, the patient should partake in a gluten-free diet for 6 months and retake the test. The absorbance readings can then be compared to indicate if the gluten-free diet had a measurable effect on the level of anti-tTG in the blood. An observable change in anti-tTG presence is a positive indicator for Celiac Disease.

This test should not be take while already on a gluten-free diet, as anti-tTG will not be produced, so the alternate case is not applicable.

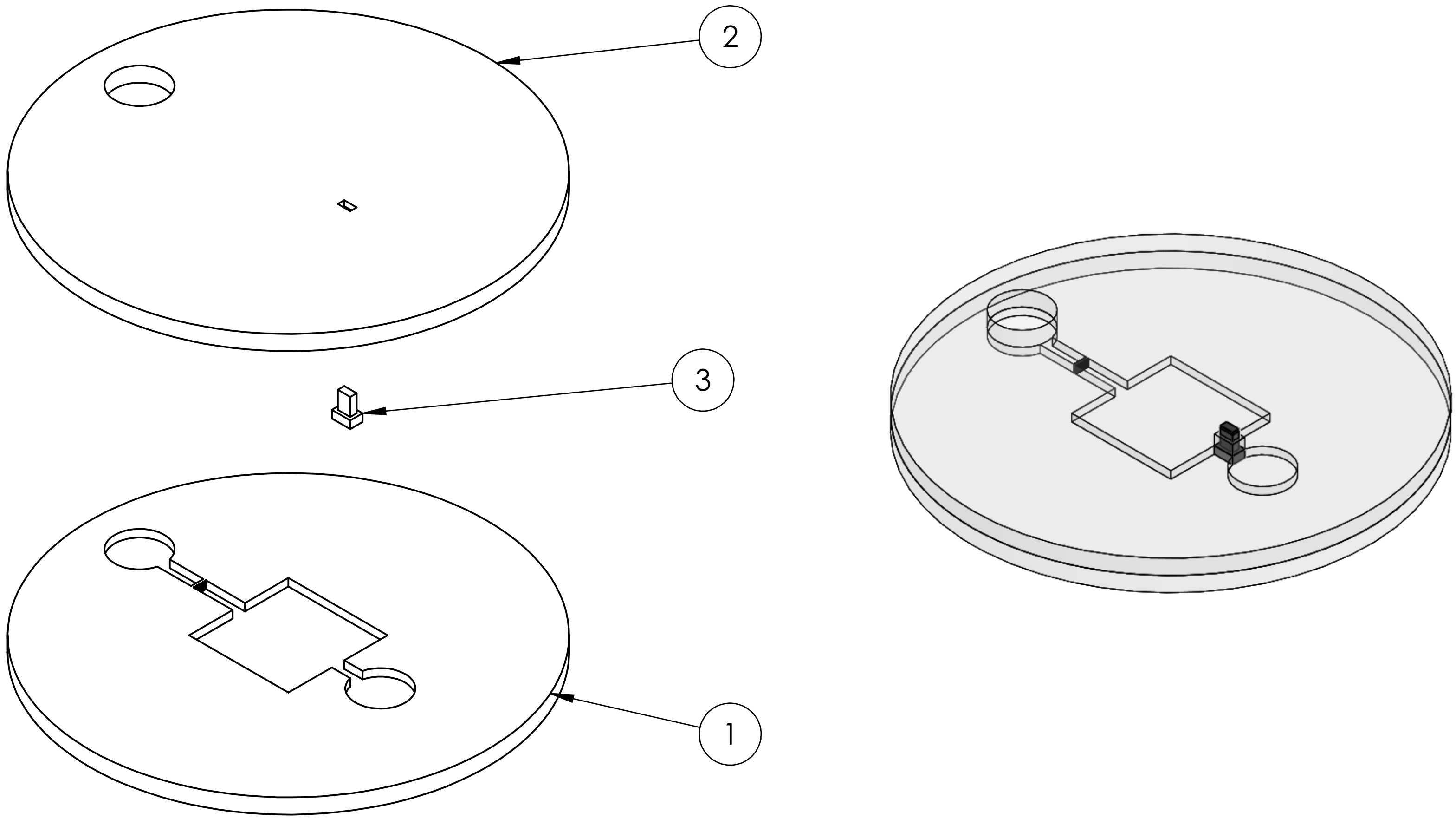
## IV. Drawings of the NANOLYSER

| Drawing Number | Description |
|---|---|
| 1 | Overall Chip |
| 2 | Chip Top Half |
| 3 | Chip Bottom Half |
| 4 | Rubber Pull Valve |
| 5 | Blood Filter |

Please refer to the end of this document for the detailed drawings.

**V. Bill of Materials**

| Material | Source | Item # |
| --- | --- | --- |
| tTG | International Scientific and Surgicals | |
| Sodium hydroxide | Carolina Biologicals | 889425 |
| Sodium salicylate | Sigma | S2679-100G |
| Sodium nitroferricyanide | Sigma | 228710-5G |
| 6% sodium hypochlorite (bleach) | Parchem | 316965017 |
| Distilled water | | |
| Wheat gliadin | Sigma | G5792_SIGMA |
| Blood filter | Fisher scientific | |




| ITEM NO. | ITEM NAME | MATERIAL | QTY. |
|---|---|---|---|
| 1 | Chip Bottom | Glass or Acrylic | 1 |
| 2 | Chip Top | Glass or Acrylic | 1 |
| 3 | Pull Valve | Rubber | 1 |



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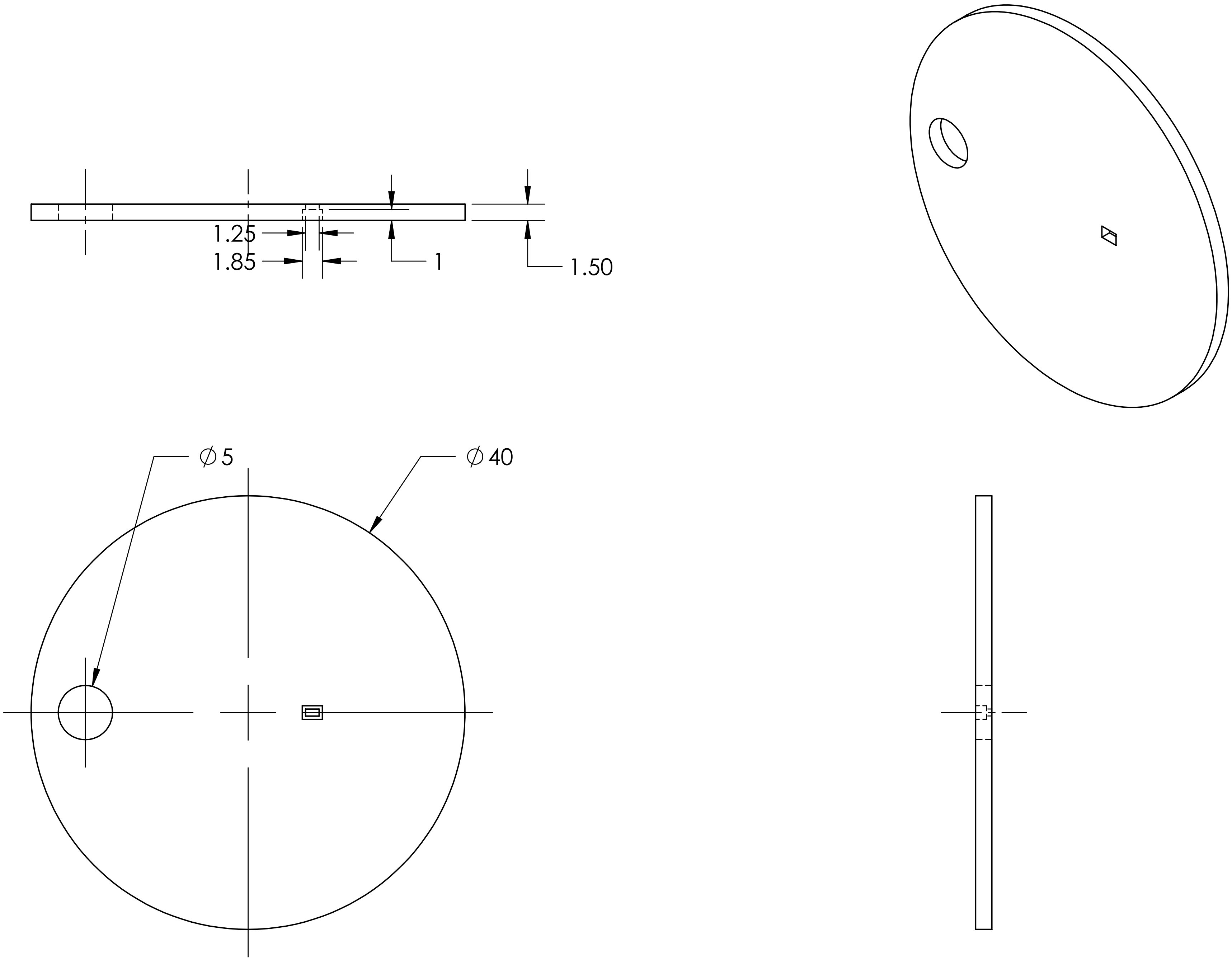
|---|---|---|---|---|
| The Ohio State University<br>First Year Engineering | Dwg. Title: OVERALL CHIP | Inst.: DMG | Scale: 2:1 | Dwg. No.: 1 |
| | Drawn By: MANOTECH | Hour: 12:40 | Units: MMGS | Date: 4/5/18 |



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|---|---|---|---|---|
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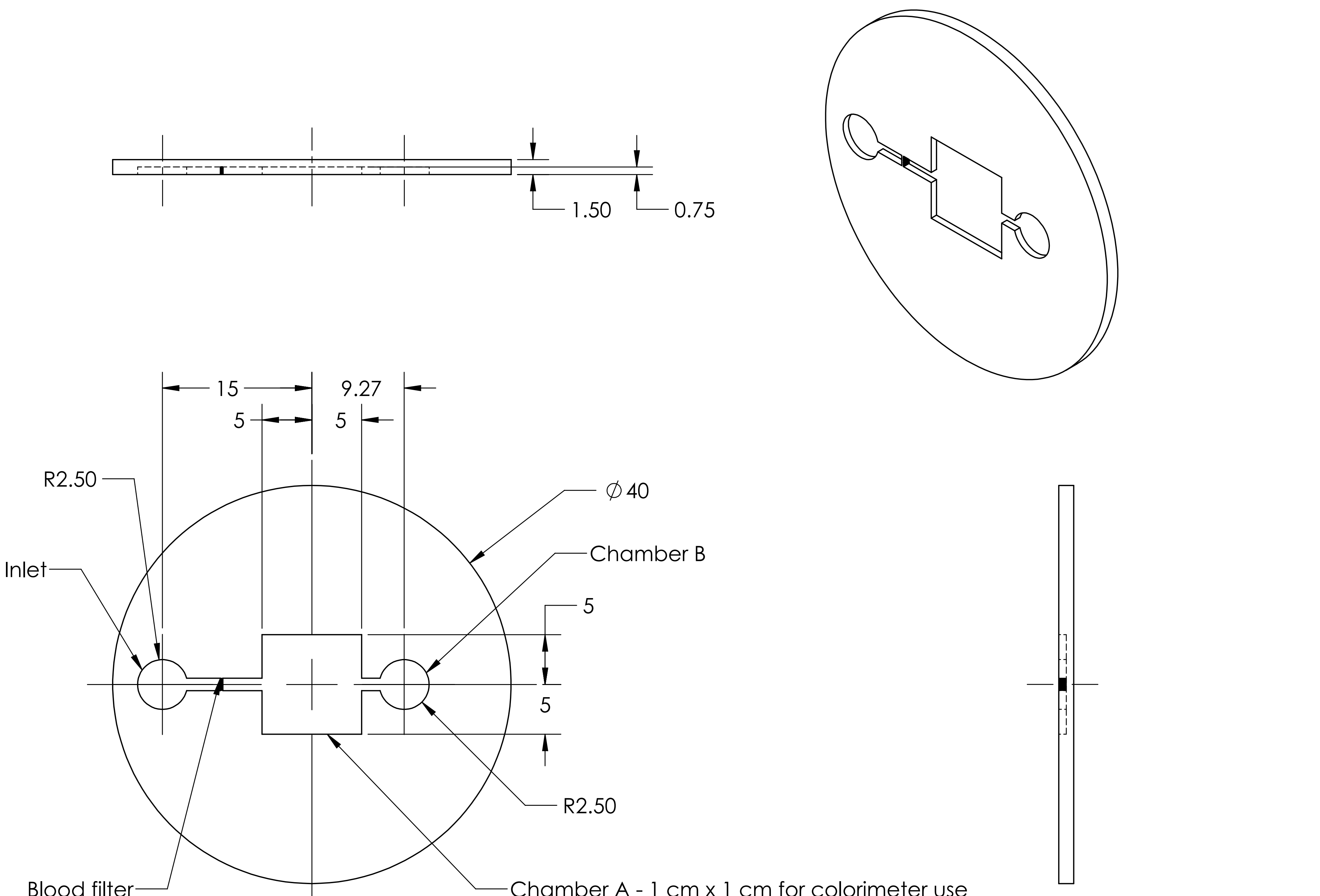




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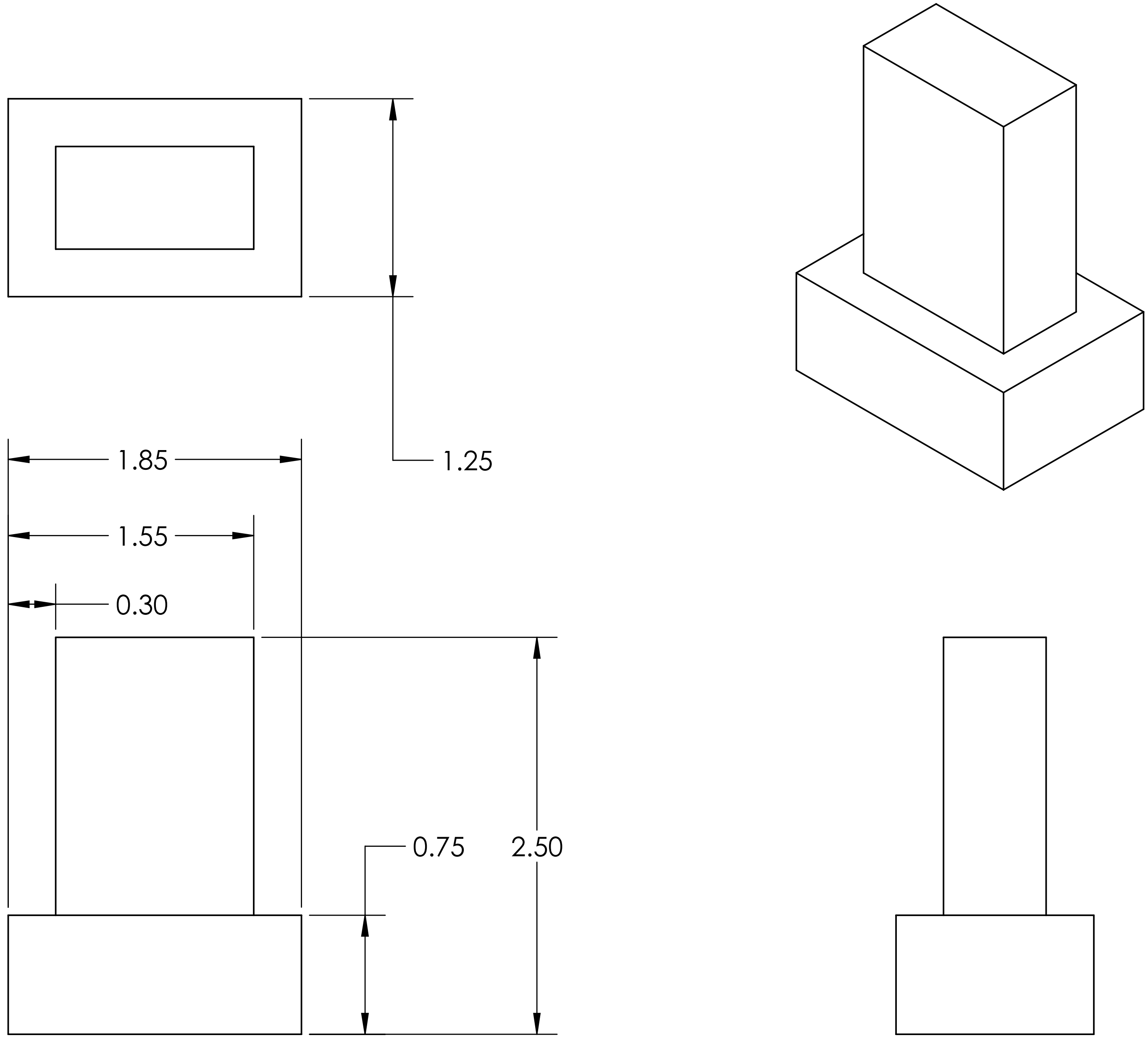
|---|---|---|---|---|
| The Ohio State University<br>First Year Engineering | Dwg. Title: CHIP BOTTOM | Inst.: DMG | Scale: 2:1 | Dwg. No.: 3 |
| | Drawn By: MANOTECH | Hour: 12:40 | Units: MMGS | Date: 4/5/18 |



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|---|---|---|---|---|
| The Ohio State University<br>First Year Engineering | Dwg. Title: PULL VALVE | Inst.: DMG | Scale: 25:1 | Dwg. No.: 4 |
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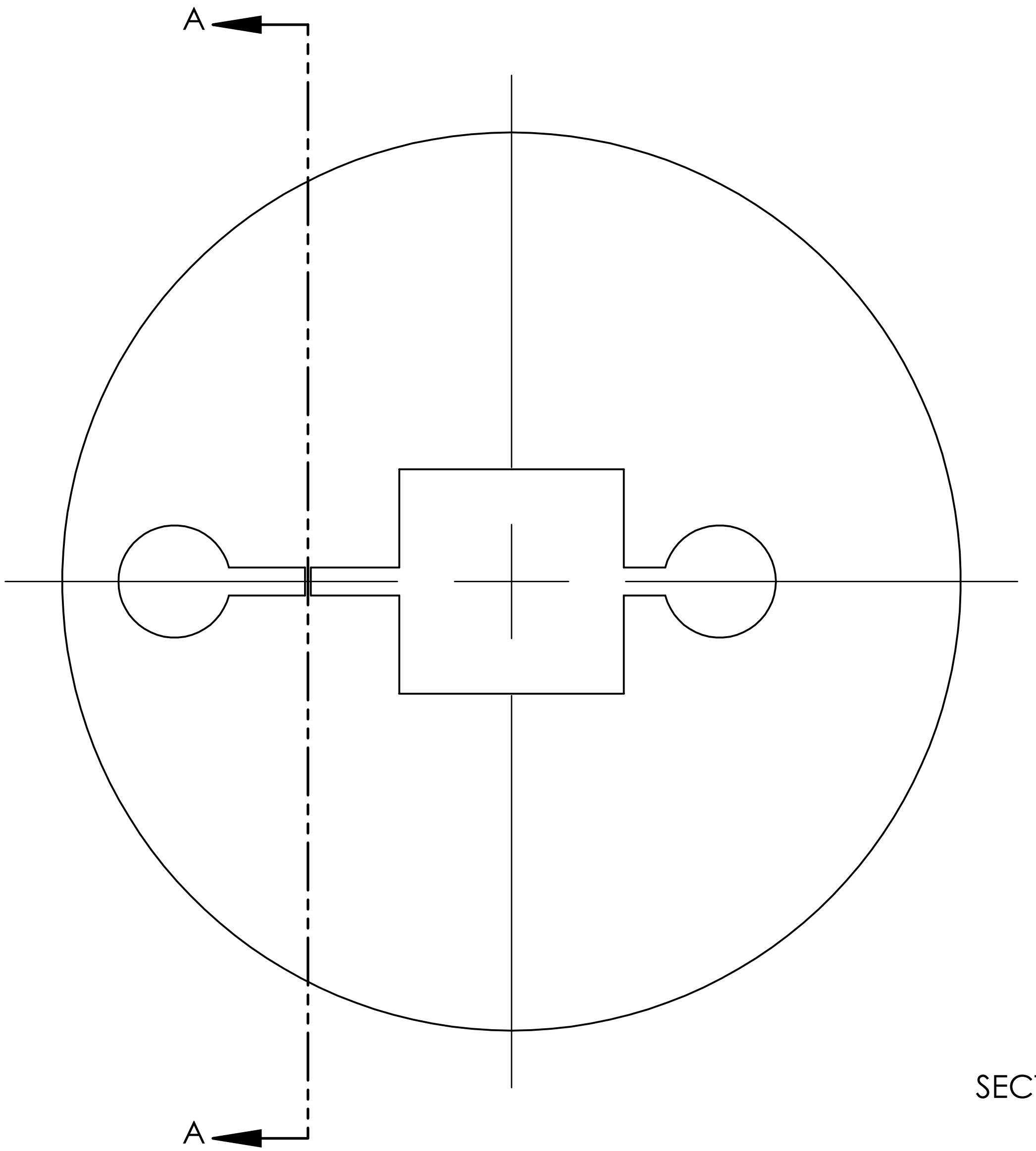
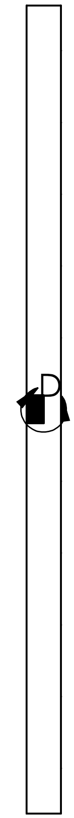
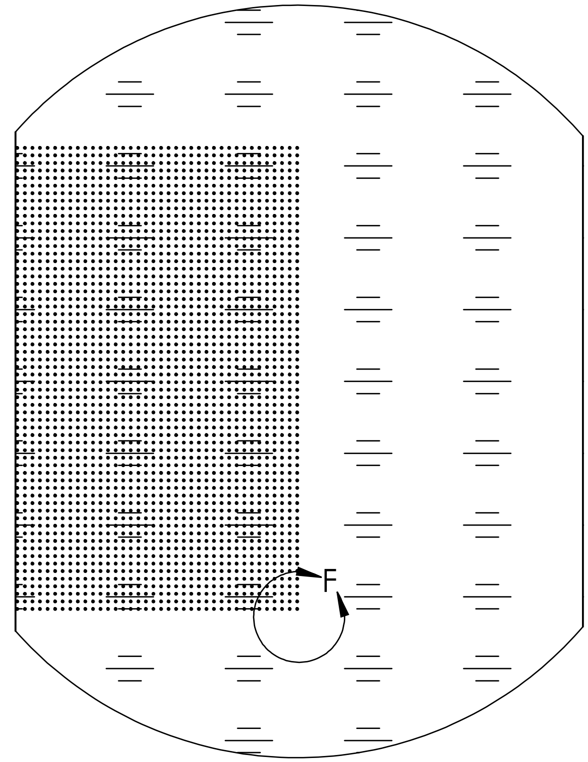
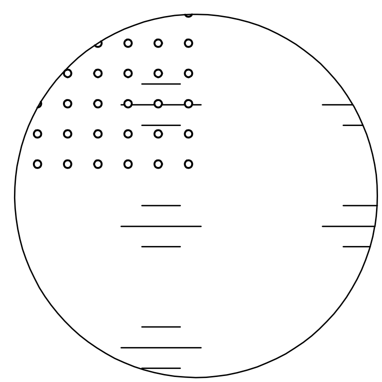


SECTION A-A




DETAIL D
SCALE 50 : 1



DETAIL F
SCALE 200 : 1



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|---|---|---|---|---|
| The Ohio State University<br>First Year Engineering | Dwg. Title: BLOOD FILTER | Inst.: DMG | Scale: 3:1 | Dwg. No.: 5 |
| | Drawn By: MANOTECH | Hour: 12:40 | Units: MMGS | Date: 4/5/18 |