Endoparasite home-test

For horses

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Contents

Problem

- Parasitic infections in horses
  - Can cause health, growth and weight loss problems
- Preventative treatment
  - Antibiotics every 6 weeks
    - Parasites develop resistance
    - Damaging to health
  - Antibiotics are cheaper than testing

Endoparasites

- Common parasites in horses
- 4 types of intestinal parasites investigated
  - Roundworms (Ascarids)
    - 90-100 microns in diameter
  - Bloodworms (Strongyles Vulgares)
    - 60-120 X 35-60 microns
  - Threadworm (Nematodes)
    - 40-52 X 32-40 microns
  - Pinworms (Oxyuris Equi)
    - 85-95 X 40-45 microns

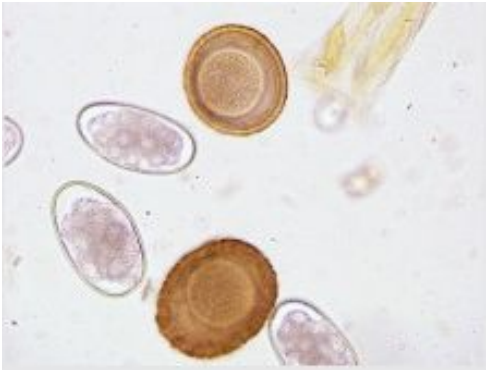


Figure 1: Strongyl (purple) and ascarids (orange) eggs [1]

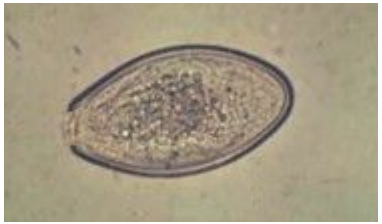


Figure 2: Pinworm egg [1]

Laboratory test

- Current method to determine if a horse is infested with worms or not
  - Fecal sample is sent to veterinary lab
  - Eggs of parasite in the feces are examined to determine if the horse is infested
  - Eggs detected with the floating technique [2]
    - Fecal sample added to a floatation solution
      - Commonly used floatation fluids are:
        - Saturated sodium chloride (NaCl; SG 1.18)
        - Sugar (Sheather's solution; SG 1.27 to 1.33)
        - Sodium nitrate (NaNO3; SG 1.18 to 1.20)
        - Magnesium sulfate (MgSO4; SG 1.20),
        - Zinc sulfate (ZnSO4; SG 1.20)
      - Eggs have a lower density (1.05 - 1.23) → float
      - Investigated under microscope → eggs are counted per species

Laboratory test

- Downsides:
  - More expensive than preventive treatment
  - Horse owner has to wait for results
  - Does not work very well for all parasitic eggs
  - The longer the determination takes the longer the horse is exposed to the parasites

Lab-on-a-chip

- Test performed at home
- No skills necessary to perform the test
- Horse owners know the result in a few minutes
- Less expensive than the treatment due to bulk production
- Manageable dimensions

Requirements

1. Determine if the horse is sick
2. Accuracy > 90%
3. The device should cost less than current treatment
4. Lab-on-a-chip
    - Horse owner can perform the test at home
5. Distinguish between different parasites
6. Determine severity of infection
7. Intuitive usage
8. Maximum area: 8.6 cm^2
    - A third of half a wafer, tests can be done in triplo

Separation

- Pinched flow fractionation (PFF)
  - Two liquids as input
    - Liquid without particles has a higher flow rate
  - Small pinched segment
    - 4 times the radius of the biggest particle
      - 240 µm
  - Broadened segment → particles separate
    - Preferred angle with pinched part = 180°

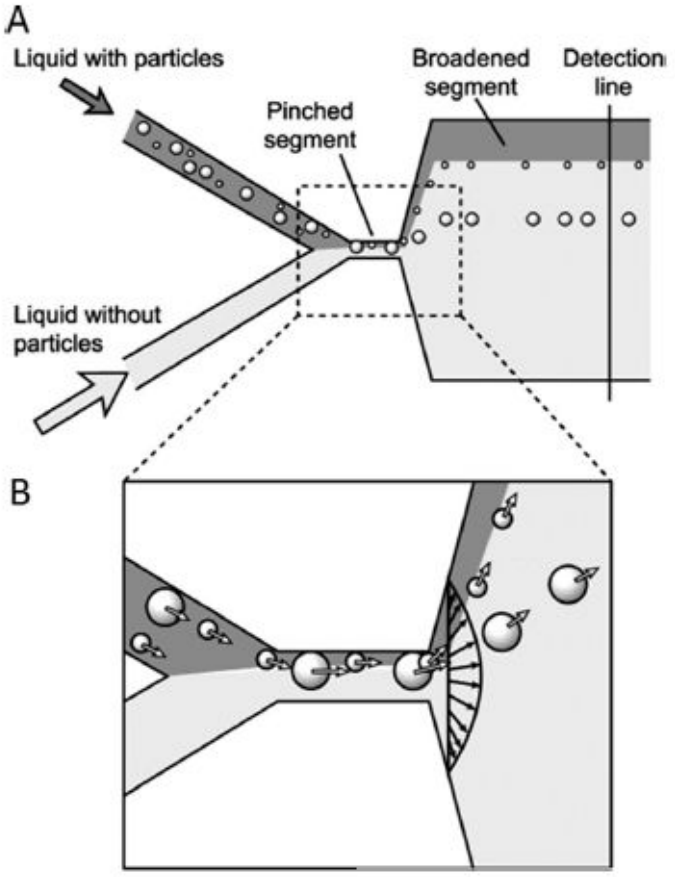


Figure 3: Schematic drawing of the PFF. From Yamada et al. *Anal. Chem.* 2004 [3]

Separation

- Spiral flow
  - 5-loop Archimedean spiral
  - Particles find equilibrium by inertial forces
    - Ratio between net lift force and Dean drag determines equilibrium position
    - Dependent on the size of the particle to the third power

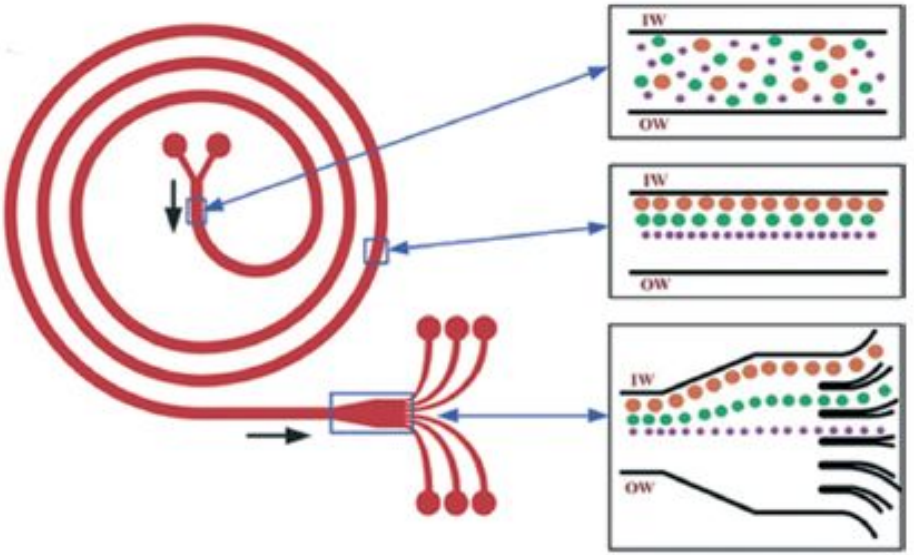


Figure 4: Schematic drawing of the spiral flow principle. From Kuntaegowdanahalli et al. *Lab chip* 2009 [4]

Filtration

- Hydrodynamic filtration
  - Done with 50 perpendicular side branch channels [5]

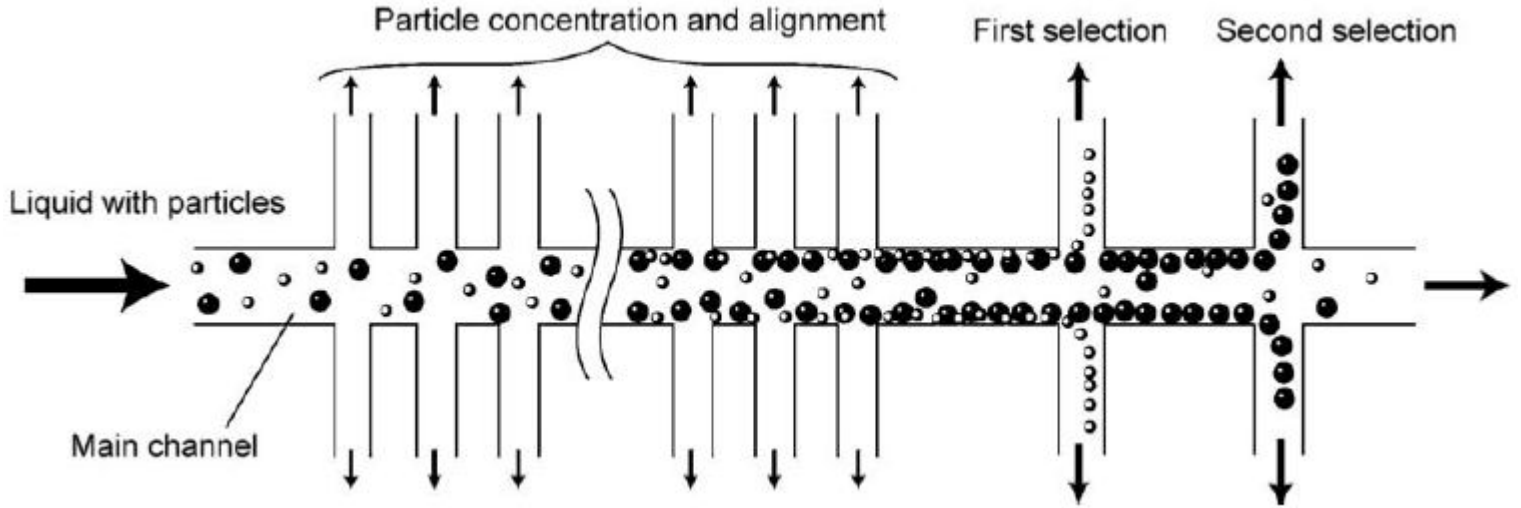


Figure 5: Schematic drawing of the hydrodynamic filtration. From Yamada et al. *Lab Chip* 2005 [5]

Filtration

- Separation part with more outlet channels than eggs counted
- Additional outlet channels for smaller and bigger particles than the eggs

→ more than 4 outlet channels

→ separation & filtration combined

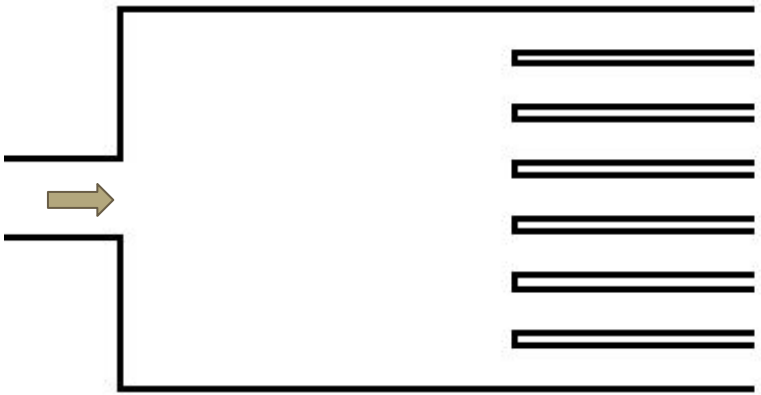


Figure 6: Sketch of pinched segment, broadening segment and the outlet channels. Arrow indicates flow direction.

Detection methods

- pH
  - Did not find relationship with parasite concentration
  - Fecal pH test already used for identifying lactose intolerance

- Color (dyes & immunoblotting)
  - Beyond scope of project and timespan
    - Parasite biochemistry

Detection methods

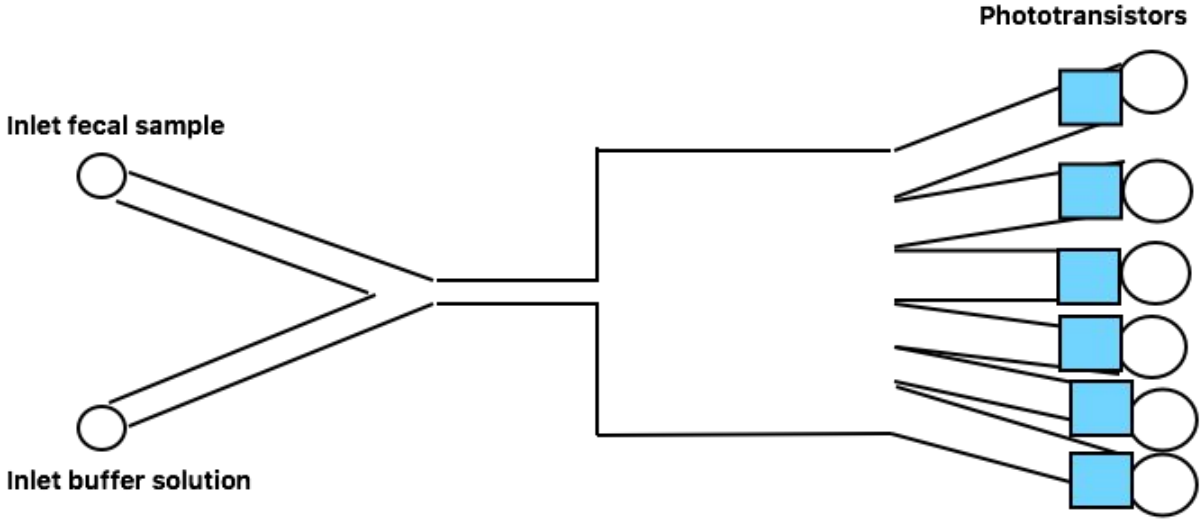
- Electrodes
  - Necessitates use of cleanroom for electrodes in micrometer scale
  - High fabrication costs
    - Use of cleanroom
    - Use of metals like platinum or gold

- Optical
  - Light sources on the bottom of the chip
    - 4 leds
    - Waveguide to focus light on the microfluidic channel
  - 4 phototransistors on the top of the chip
    - Translates light to current

Conclusion

- Pinched flow fractionation
  - Takes up less space than the spiral microchannel
    - Dimensions in mm instead of the 3x3 cm of the spiral
- Filtering by extra channels after the pinched segment
  - No separate part needs to be made
- Optical detection
  - No use of cleanroom required unlike the electrical detection

Design


Phototransistors
Inlet fecal sample
Inlet buffer solution

Back-up

- Test the hydrodynamic filtration separately as back-up for the whole chip
  - Different resistances to separate eggs also from each other
  - Optical counting
- Test the spiral separately with smaller dimensions
  - Compare the performance of the spiral with the PFF
- For detection: use the microscope to count

Experimental methods

- Verify the number of eggs counted by counting under the microscope
- Look at how many different eggs are in one channel (microscope)
    - Amount of debris can also be looked at

References

[1] http://eggzamin.com/for-the-vet/the-parasites/
[2] http://www.vet.utk.edu/diagnostic/parasitology/Detections%20of%20Parasitic%20Infections%20by%20Fecal%20Exam.pdf
[3] Yamada M, Nakashima M, Seki M. Pinched Flow Fractionation: Continuous Size Separation of Particles Utilizing a Laminar Flow Profile in a Pinched Microchannel. Analytical Chemistry. 2004;76(18):5465–5471.
[4] Kuntaegowdanahalli SS, Bhagat AAS, Kumar G, Papautsky I. Inertial microfluidics for continuous particle separation in spiral microchannels. Lab Chip. 2009;9:2973–2980.
[5] Yamada M, Seki M. Hydrodynamic filtration for on-chip particle concentration and classification utilizing microfluidics. Lab Chip. 2005;5:1233–1239.