

The background is a light blue gradient with several realistic water droplets of various sizes scattered across it. The droplets have highlights and shadows, giving them a three-dimensional appearance.

# THE EFFECTS OF WATER CONTAMINANTS ON THE IMMUNE SYSTEM OF FATHEAD MINNOWS

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RESEARCH ADVISOR: DR. MARINA CETKOVIC-CVRLJE

# WHAT ARE CEC?

- CONTAMINANTS OF EMERGING CONCERN (CEC) - CHEMICAL COMPOUNDS FOUND IN THE ENVIRONMENT IN CONCENTRATIONS THAT COULD POTENTIALLY BE HIGH ENOUGH TO DISRUPT SOME COMPONENT OF AQUATIC LIFE IN A NEGATIVE WAY.
- THE EPA GUIDELINES FOR ADDRESSING CEC SINCE 1985.
- NANOPARTICLES, PHARMACEUTICALS, PERSONAL-CARE PRODUCTS, ESTROGEN-LIKE COMPAOUNDS, FLAME RETARDANTS, DETERGENTS, AND SOME INDUSTRIAL CHEMICALS



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<http://www.startribune.com/deformed-frog-resources/169510706/>

# HOW ARE CEC INVESTIGATED?

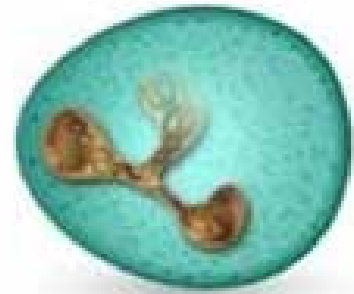
- FATHEAD MINNOWS (*PIMEPHALES PROMELAS*) ARE COMMONLY USED AS A MODEL ORGANISM DUE TO ITS STATUS AS A HABITAT GENERALIST.
- CEC EFFECTS ON BLOOD PARAMETERS, TISSUE GROWTH AND COMPOSITION, GENE EXPRESSION, AND MULTIPLE BEHAVIORS SUCH AS NEST DEFENSE, PREDATORY AVOIDANCE, AND REPRODUCTIVE SUCCESS.
- GAP IN KNOWLEDGE ON THE EFFECTS OF CEC IN THE IMMUNE SYSTEM OF FATHEAD MINNOW MODEL ORGANISM.



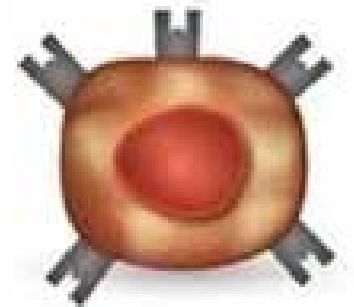
Image retrieved from: <https://www.smithcreekfishfarm.com/new-york-hatchery/fathead-minnows-for-sale.html>

# THE IMMUNE SYSTEM – INNATE AND ADAPTIVE.

- INNATE IMMUNITY - A NON-SPECIFIC RESPONSE THAT CONSISTS OF ALL CELLS, PROTEINS, AND STRUCTURES THAT PREVENT EXPOSURE TO A PATHOGEN OR ADDRESS THE PATHOGEN IMMEDIATELY AFTER EXPOSURE.
- A VERY COMMON AND IMPORTANT CELL OF INNATE IMMUNITY IS THE **NEUTROPHIL**.
- ADAPTIVE IMMUNITY - CONSISTS OF THE CELLS AND MOLECULES THAT COORDINATE AN ATTACK OF A PATHOGEN WITH GREAT SPECIFICITY; LONG-TERM IMMUNITY.
- A VERY COMMON AND IMPORTANT CELL OF ADAPTIVE IMMUNITY IS THE LYMPHOCYTE **T-CELL**.



**Neutrophil**



**T Cell**

# OBJECTIVE

- DEVELOP REPRODUCIBLE ASSAYS FOR STUDYING THE FUNCTION OF THE INNATE AND ADAPTIVE IMMUNE SYSTEM IN FATHEAD MINNOWS.
- INNATE IMMUNITY ASSAY – BASED ON QUANTIFICATION OF NEUTROPHIL FUNCTION THROUGH DEGRANULATION AND MEASUREMENT OF MYELOPEROXIDASE (MPO) RELEASE.
- ADAPTIVE IMMUNITY ASSAY – BASED ON QUANTIFICATION OF T-CELL FUNCTION (T-CELL PROLIFERATION)
- TEST THE EFFECT OF COMMON CEC (ESTRADIOL) ON THE FUNCTION OF NEUTROPHILS AND T-CELLS USING THESE ESTABLISHED ASSAYS.

# INITIAL CHALLENGE FOR BOTH ASSAYS?

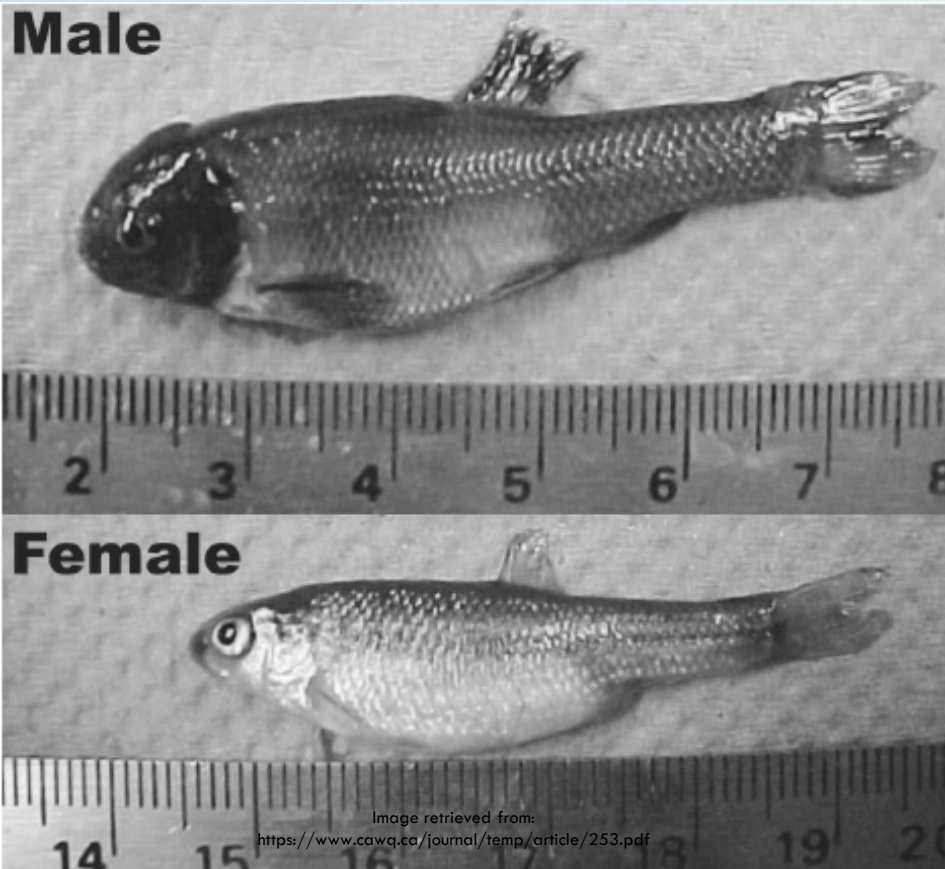




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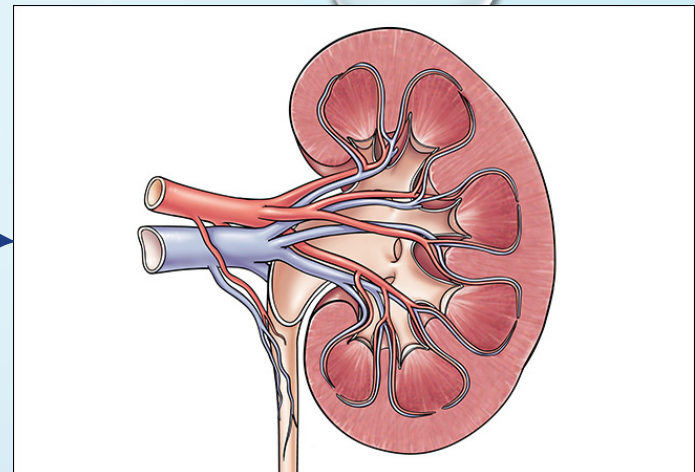


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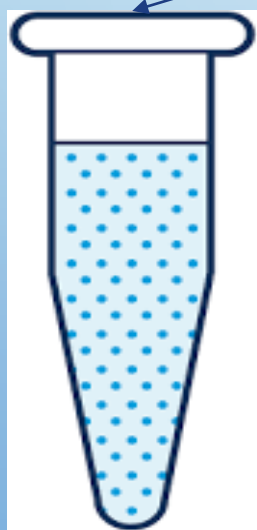


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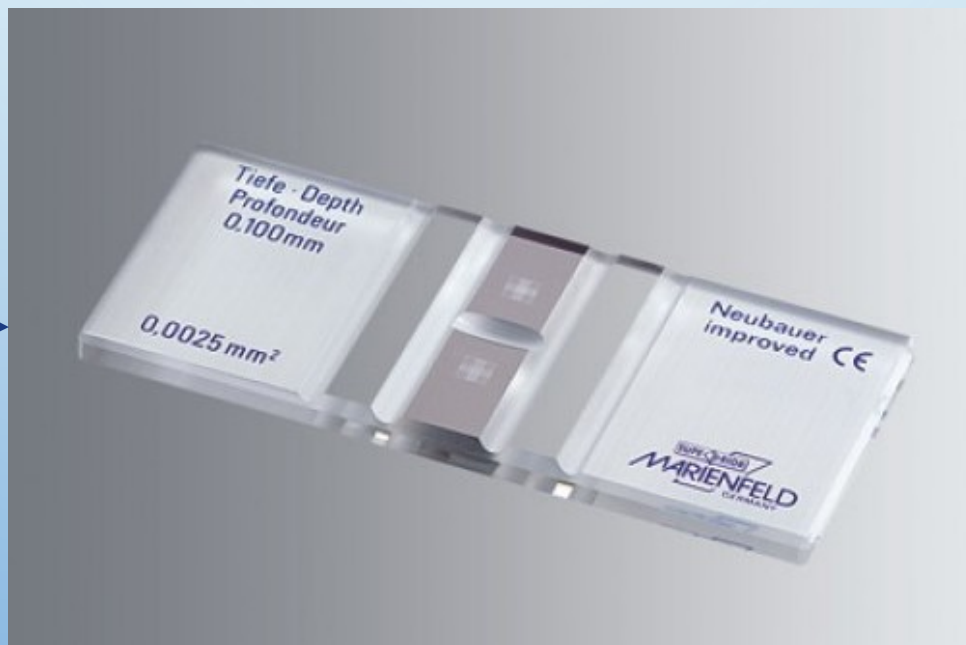


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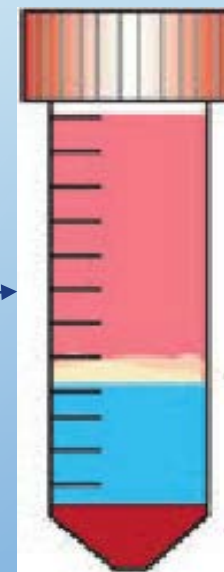


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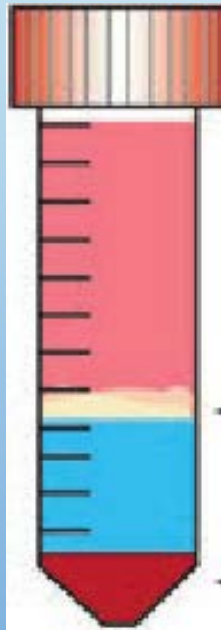


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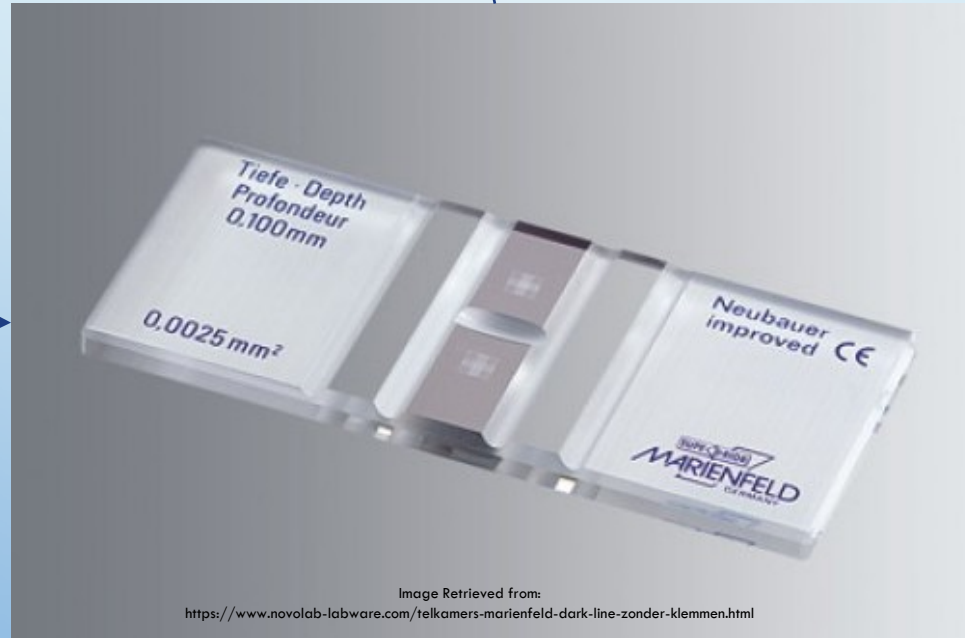
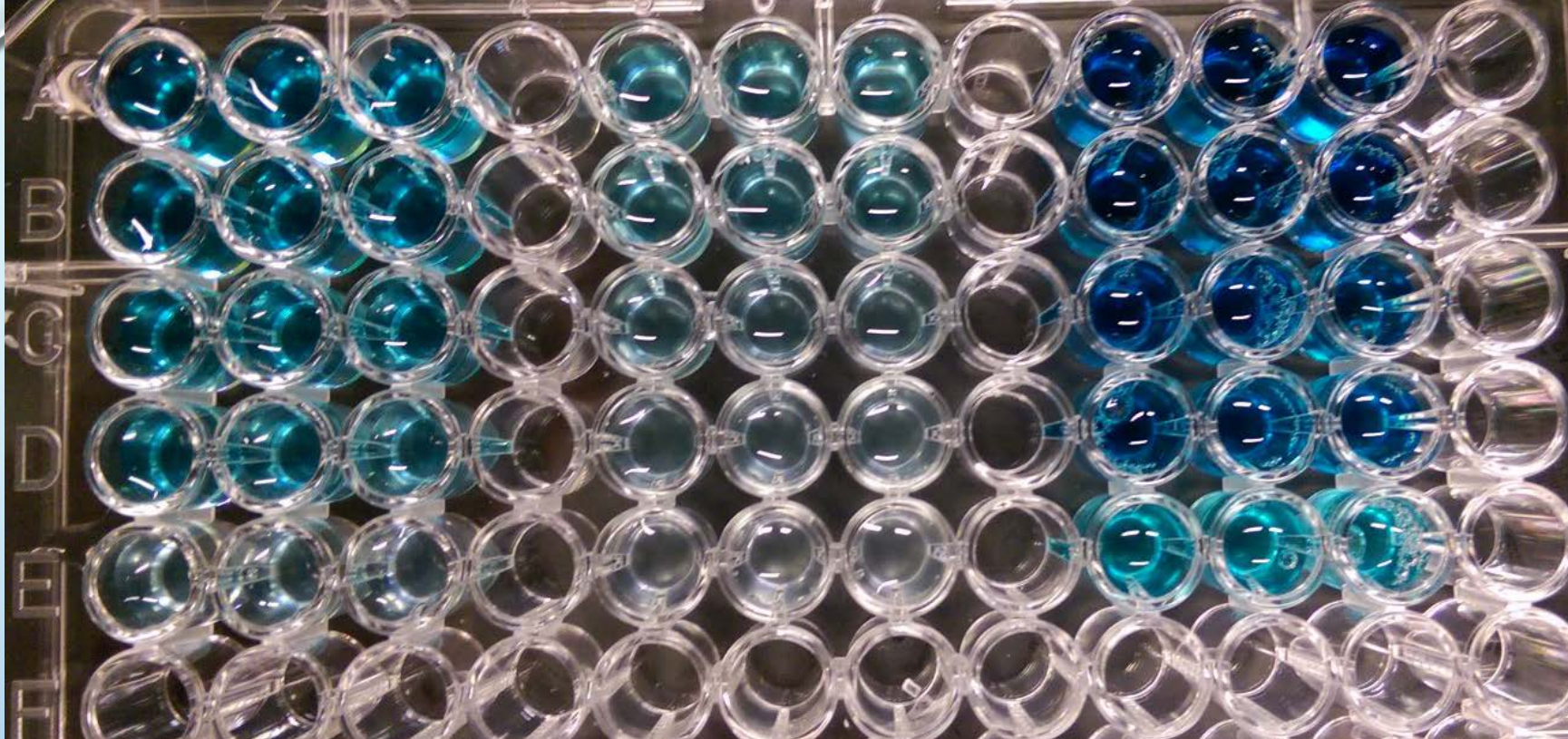


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- DEGRANULATION – ARTIFICIALLY STIMULATED BY CALCIUM IONOPHORE
- MYELOPEROXIDASE (MPO) – RELEASED DURING DEGRANULATION
- QUANTIFICATION OF MPO RELEASE THROUGH BINDING TO 3,3',5,5'-Tetramethylbenzidine.



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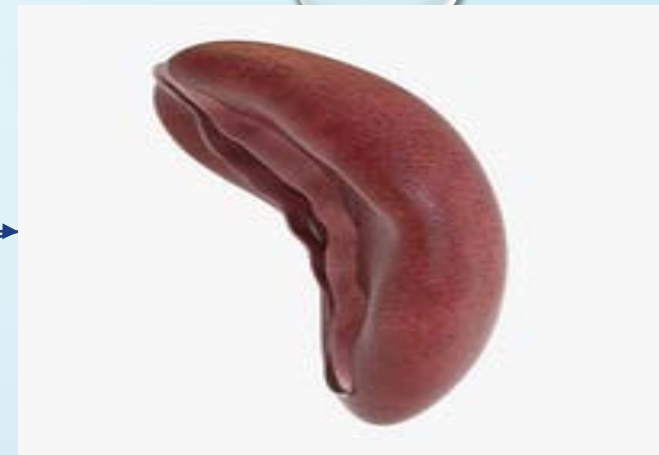


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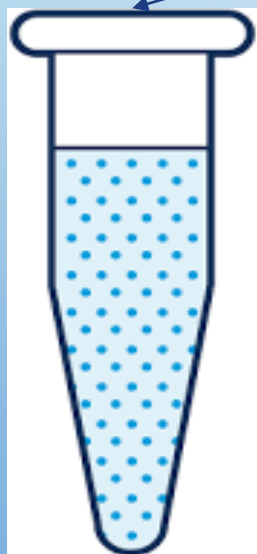


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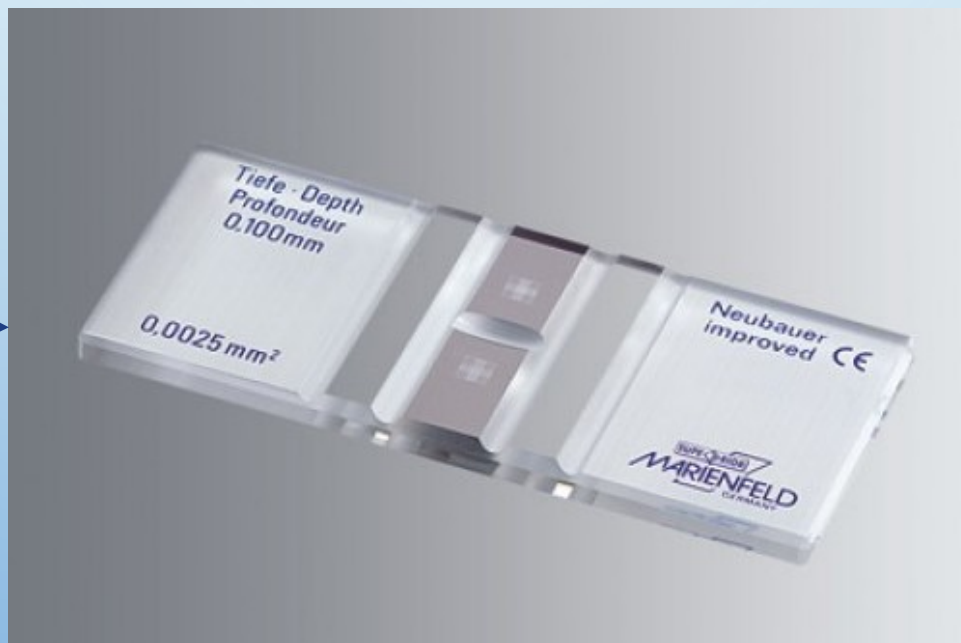


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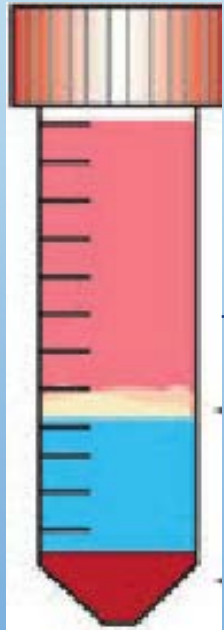


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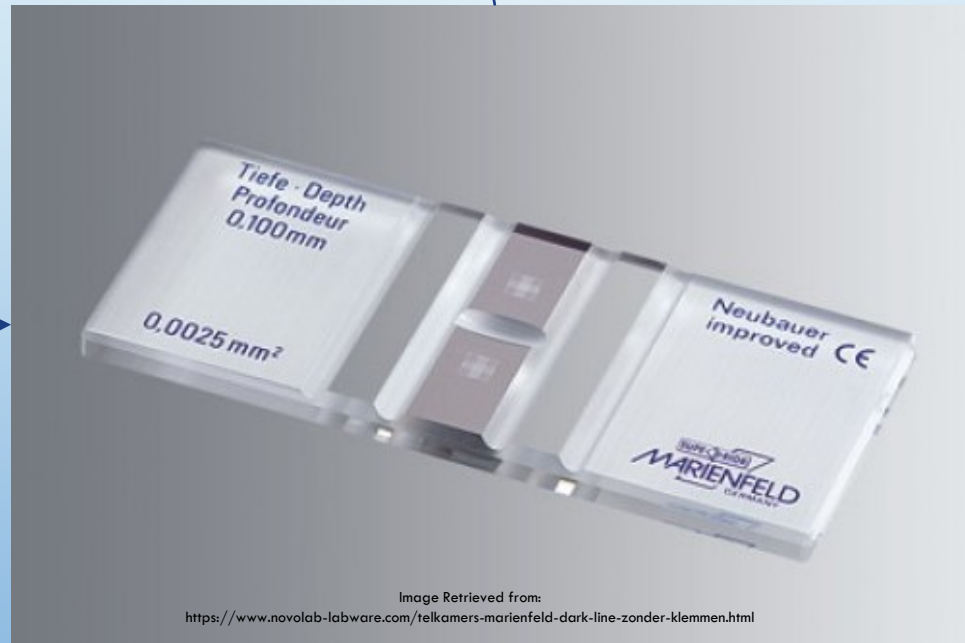
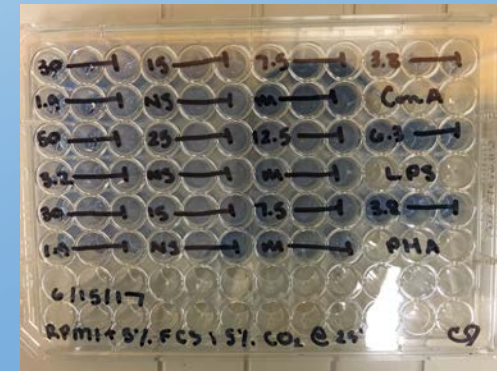


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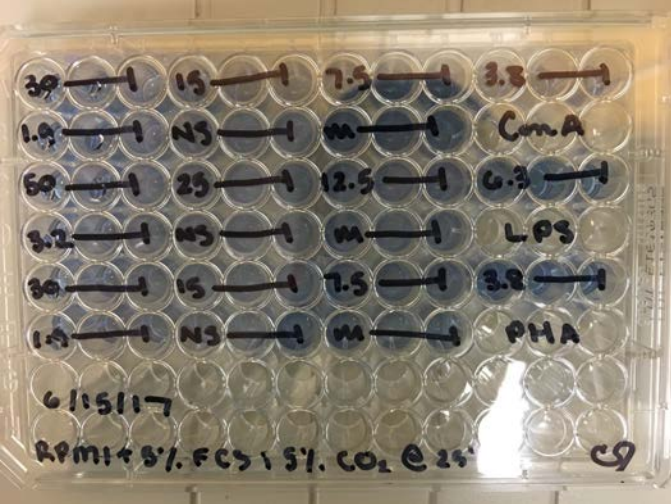


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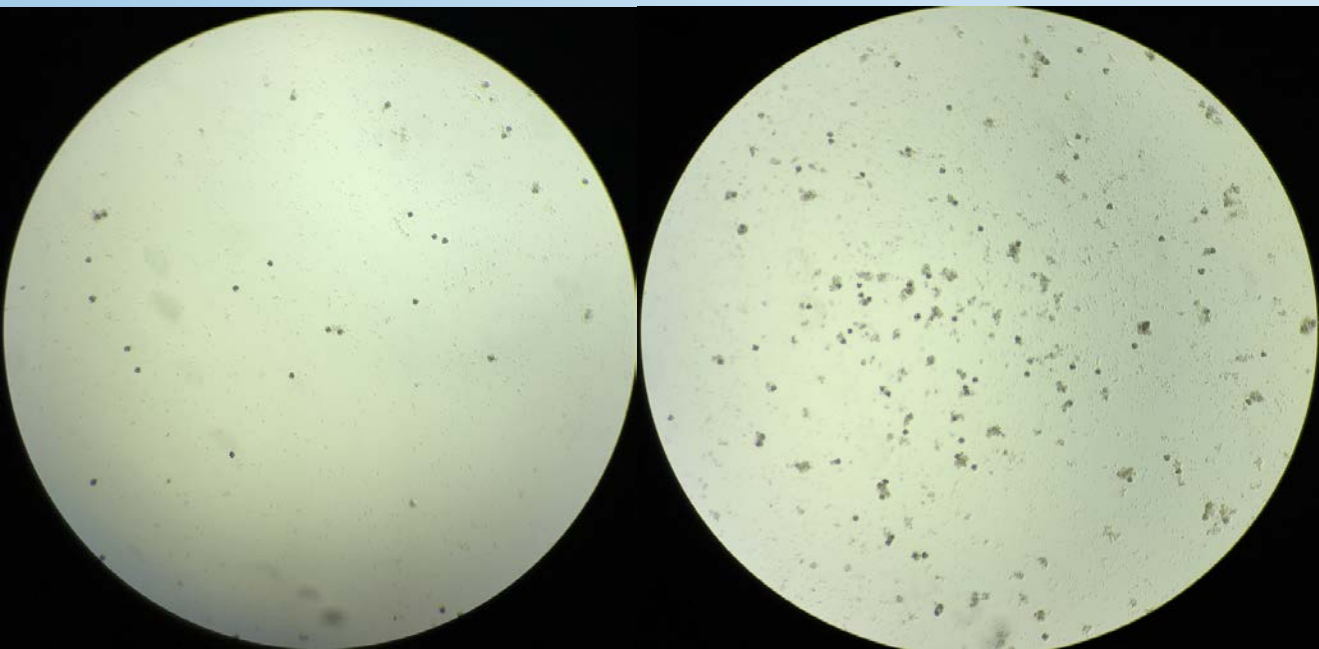
Plate reader  
 (570 nm)



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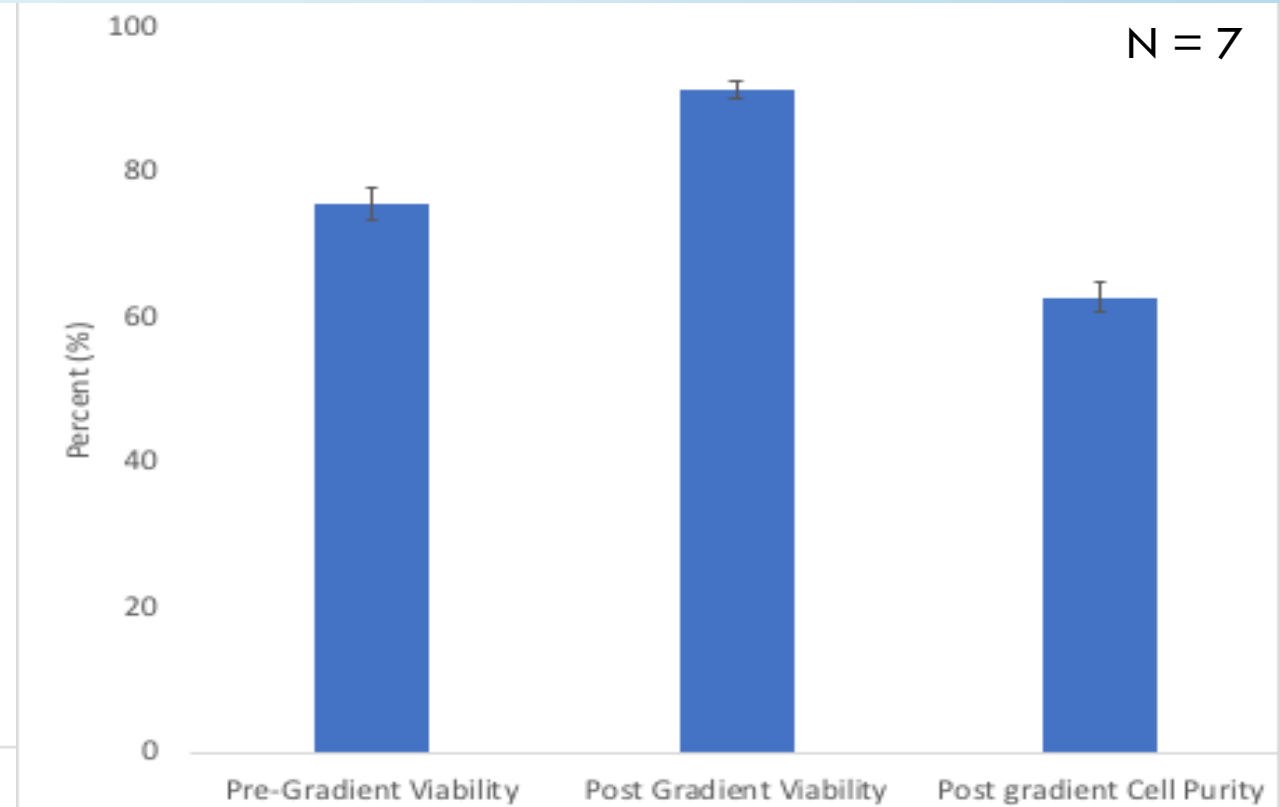
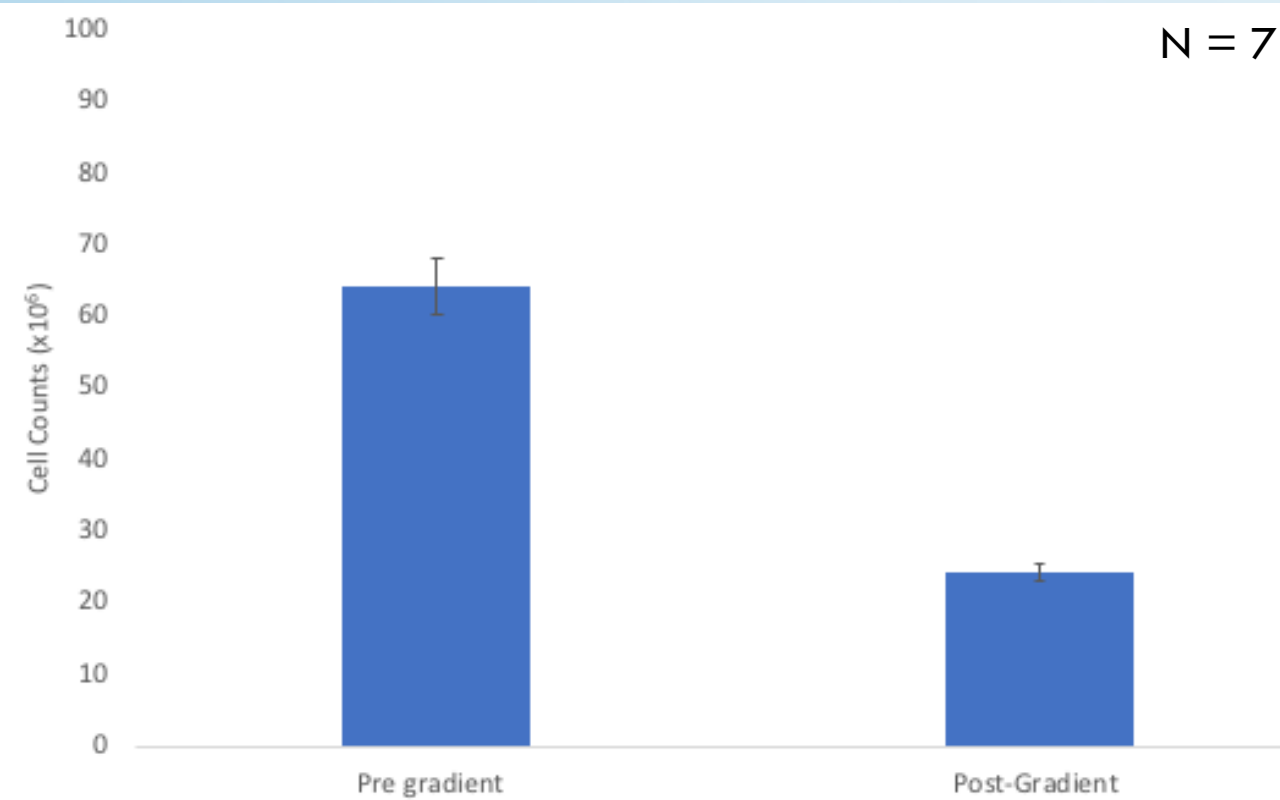
Cells in a non-stimulated control well

Cells in a stimulated well.  
 (3.8  $\mu$ g/mL ConA in RPMI)

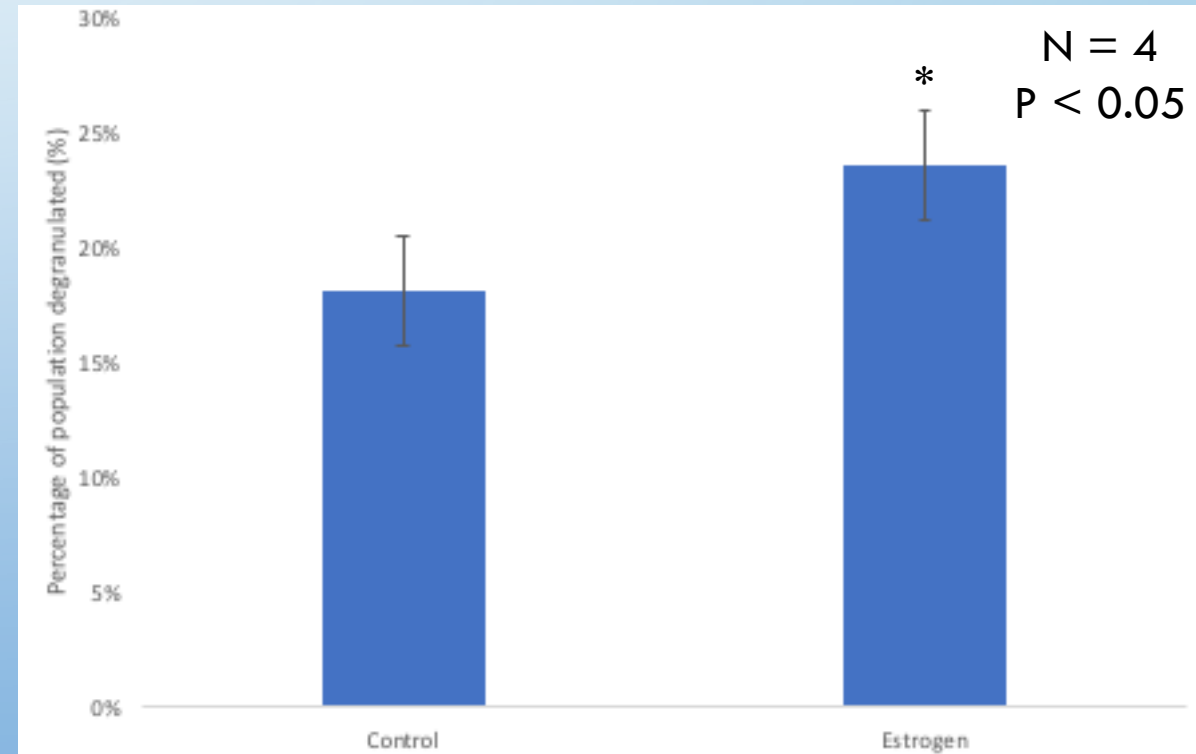
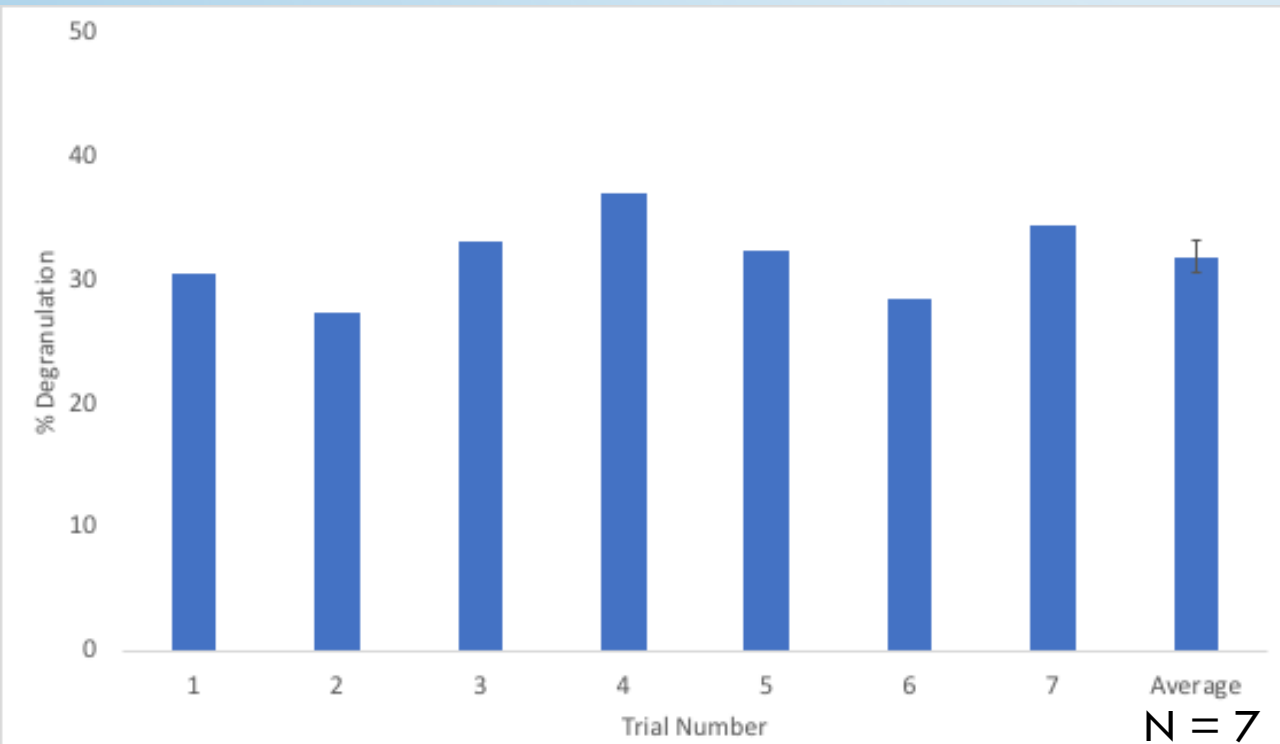




# NEUTROPHILS: ISOLATION, CELL COUNTS, VIABILITY & PURITY



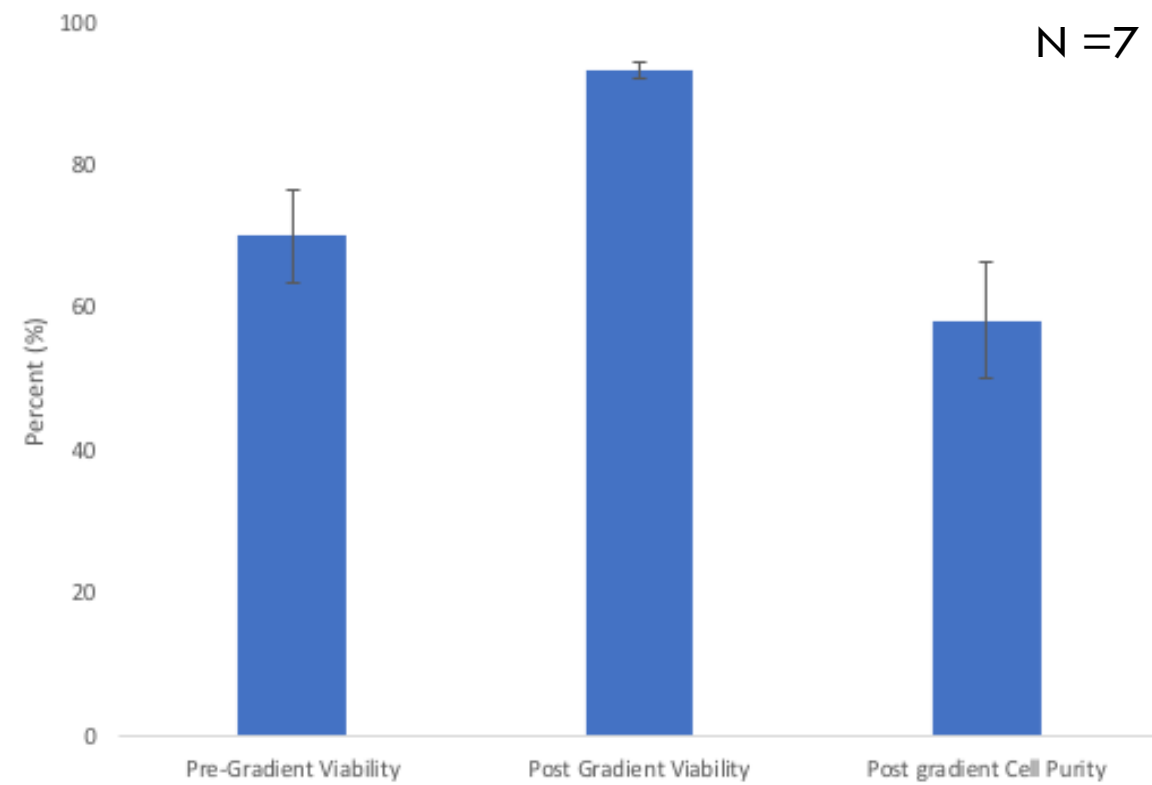
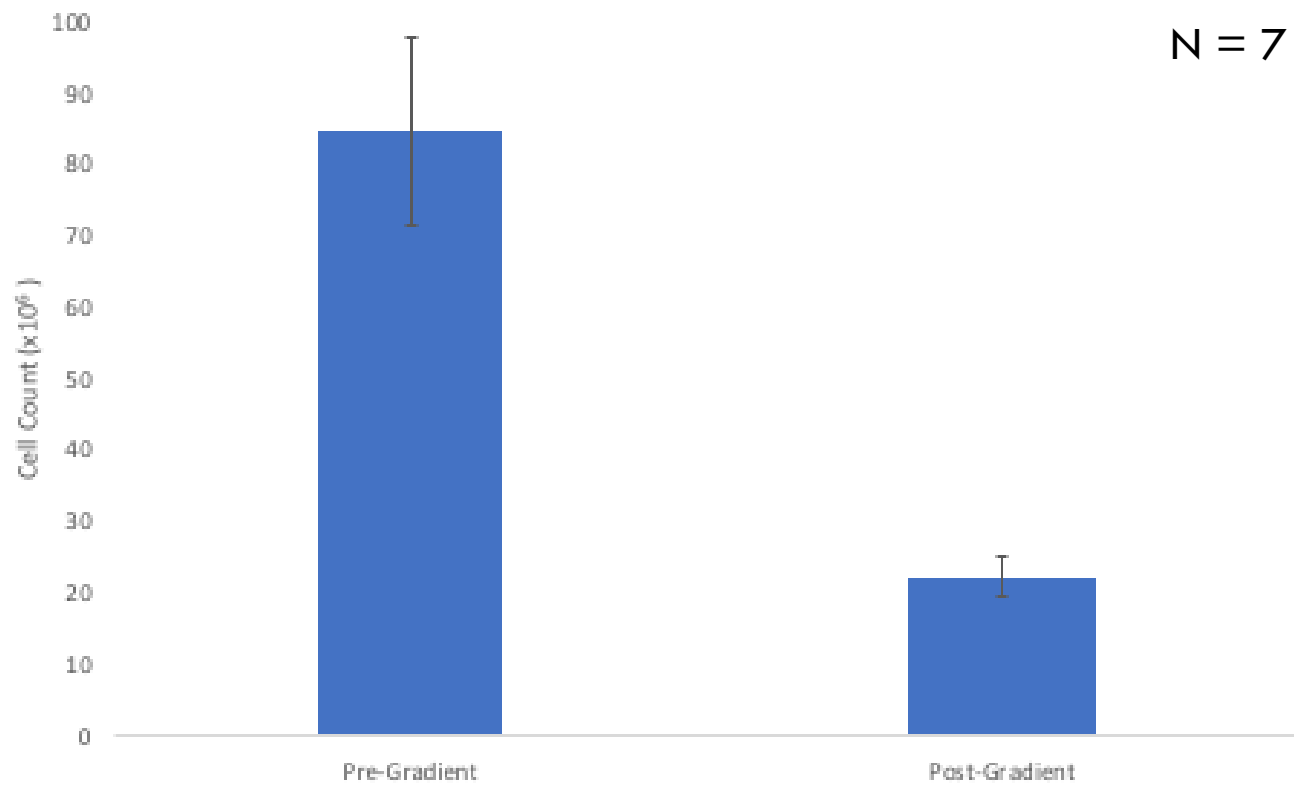
# NEUTROPHIL DEGRANULATION ASSAYS





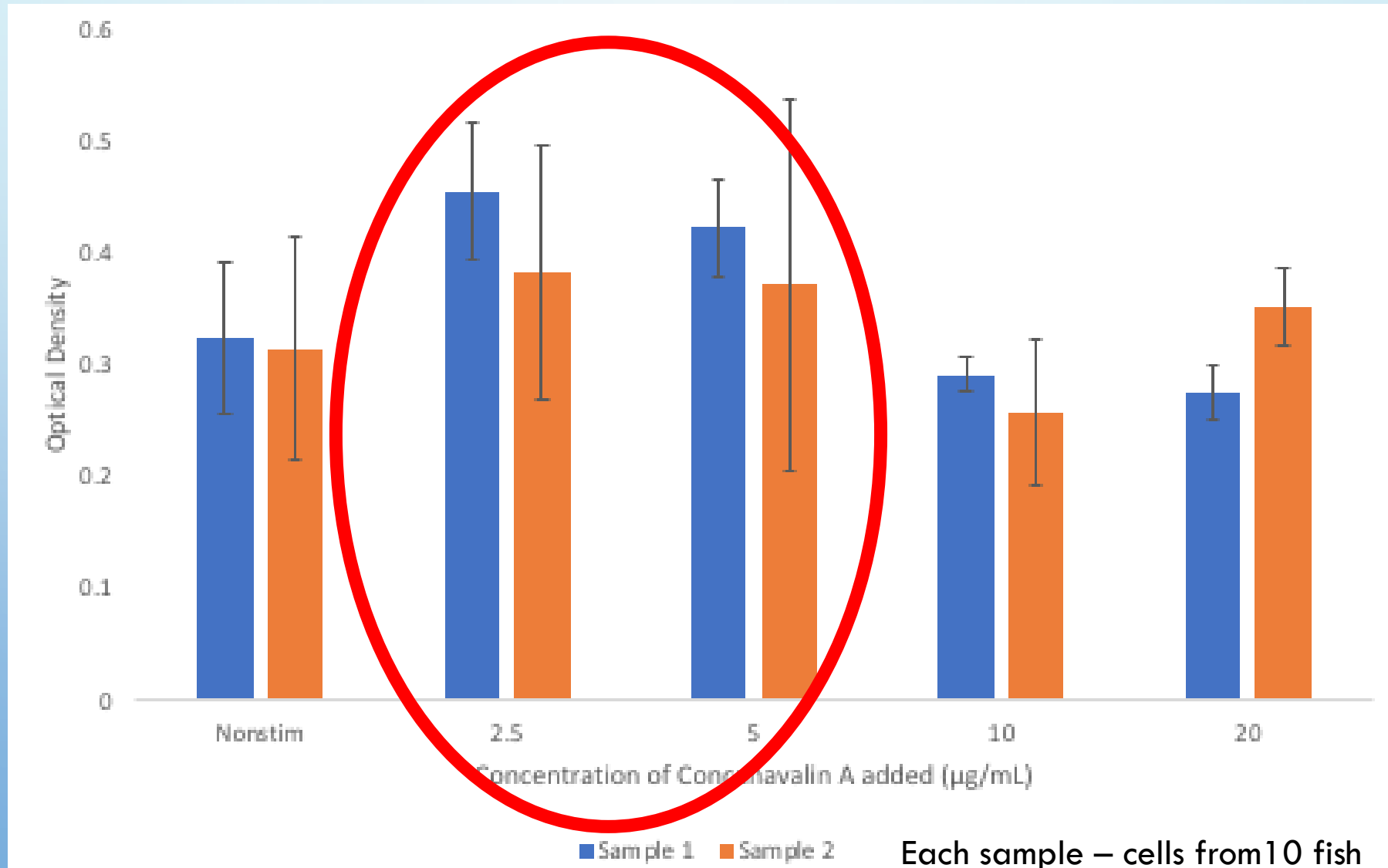
# LYMPHOCYTES

## ISOLATION CELL COUNTS, VIABILITY & PURITY



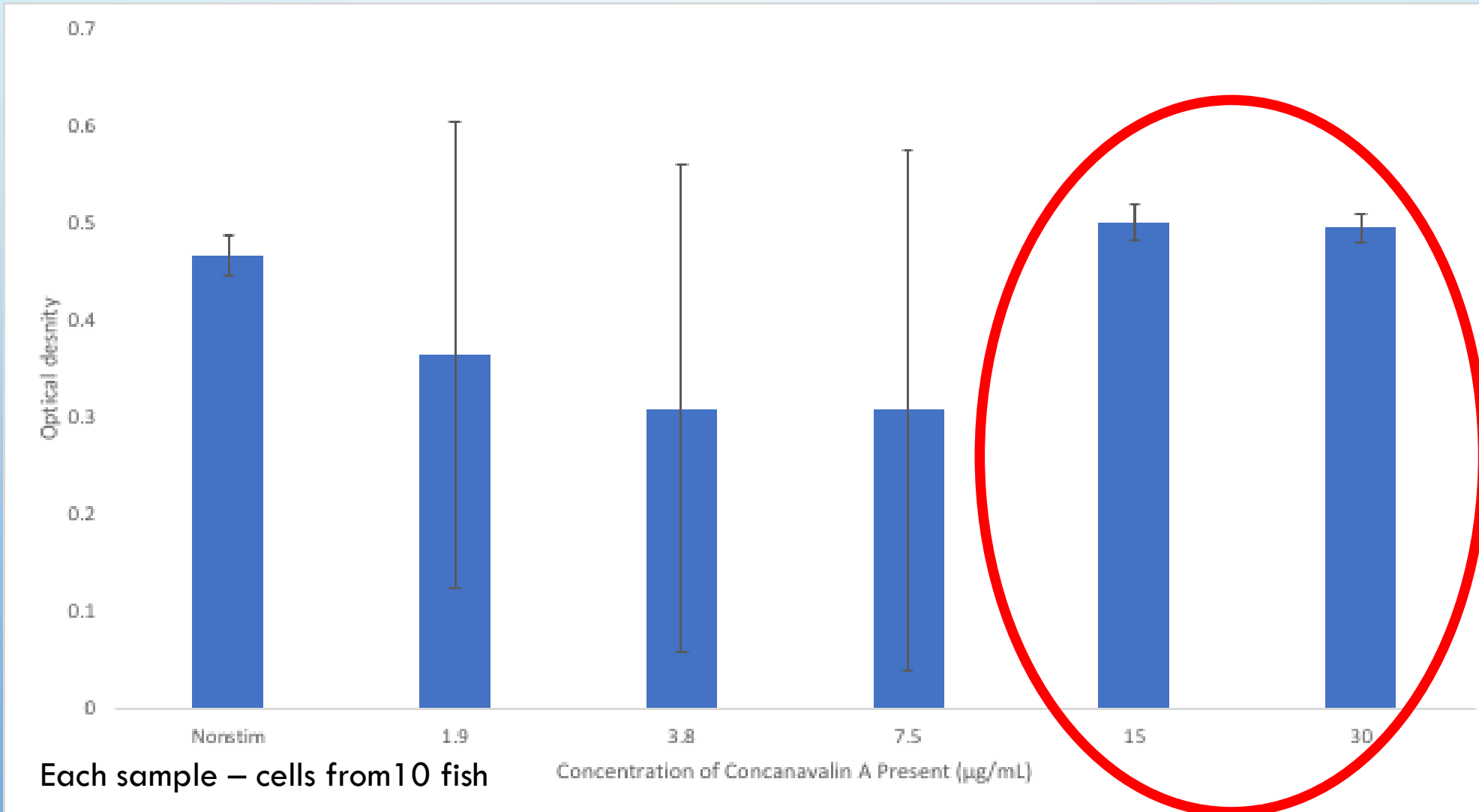
# T-CELL PROLIFERATION ASSAY

(RPMI, 26°C, 5% CO<sub>2</sub>, 5 DAYS)



# T-CELL PROLIFERATION ASSAY

(L-15, 25°C, 5 DAYS)



# SUMMARY

- FATHEAD MINNOW SPLEEN CONFIRMED AS THE APPROPRIATE ORGAN TO OBTAIN T-CELLS
- REPRODUCIBLE FUNCTIONAL ASSAY FOR EVALUATION OF T-CELL FUNCTION NOT ESTABLISHED
- ANTERIOR KIDNEY CONFIRMED AS THE APPROPRIATE ORGAN TO OBTAIN NEUTROPHILS
- REPRODUCIBLE MPO NEUTROPHIL ASSAY ESTABLISHED
- ESTRADIOL FOUND TO INCREASE NEUTROPHIL MPO RELEASE POST ACUTE EXPOSURE

# CONCLUSION

WHEREAS FURTHER EFFORTS ARE REQUIRED TO OBTAIN FUNCTIONAL ASSAY FOR ADAPTIVE IMMUNITY CELLS, ESTABLISHED NEUTROPHIL MPO ASSAY WILL ALLOW TESTING THE EFFECTS OF CEC ON THE INNATE IMMUNE SYSTEM OF FATHEAD MINNOWS.

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