Title: Paying It Forward: Amplifying the Effects of UF HHMI ICORE Emerging Pathogens Summer Institute

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Abstract: This proposal has as its goal to amplify the effects of the HHMI ICORE Emerging Pathogens Institute by conducting a full day inservice for 28 Volusia County science teachers. The rationale for this project stems from the fact that there has been only 1 teacher from this district who has been able to attend either this institute or the biotechnology institute. This professional development day will feature: 1) An introduction to the problem of emerging pathogens 2) A hands-on introduction to micropipetting 3) An investigation of Tomato Spotted Wilt Virus using ImmunoStrip Test, DNA extraction, PCR, and gel electrophoresis. A novel aspect of the proposal includes the utilization of 12th grade students, previously exposed to this information and techniques as mentors for these teachers. Measuring success will involve tracking the number of teachers who utilize these lessons and the number of students exposed in their respective schools.

Mission Statement: To increase the awareness of emerging pathogens among teachers and students by providing a professional development opportunity for Volusia County middle and high school science teachers. Teachers, paired with 12th grade students in the International Baccalaureate (IB) Program, will have hands-on instruction and practice using micropipets, extracting and amplifying DNA, and running agarose gels using an important plant virus as a model system.

Description of Teaching Unit:

Rationale. Travelling to Gainesville and staying in a residence hall for 1 or 2 weeks can be a tremendous burden for many teachers with young families, aging parents, or second jobs. As a result, I have noticed that I have been the only teacher to attend either the Biotechnology Institute or the Emerging Pathogens Institute from Volusia or Flagler Counties. This is most probably the result of individual teacher responsibilities rather than disinterest. The way the proverb goes is: "Give a person a fish, and you feed him for a day; teach him to fish and you feed him for a lifetime." Teaching teachers is the most effective way of reaching a large number of students over a long period of time. As a result, I have chosen as my student population, middle and high school teachers in Volusia County, and, in keeping with the DNA technology being used, its purpose is to **amplify** the result of the HMMI ICORE program in Emerging Pathogens.

Information Covered

In 1992, the Institute of Medicine defined emerging infections as "New, reemerging or drug-resistant infections whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future." (Dr. Paul Gibbs, College of Veterinary Medicine, University of Florida lecture to ICORE 6/24/09). Some of these pathogens are viruses (HIV, influenza, TSWV), bacteria (tuberculosis), and protozoans (malaria). Some have other reservoirs than humans (bird flu, malaria) while others do not; some can be prevented with vaccines while others can not be. These pathogens impact animal, plant, and public health. The teaching of emerging pathogens fits well into discussions of taxonomy (as examples within the various categories of organisms), evolution, and systems of defense (including the immune system). A formal presentation will provide an overview of these diverse pathogens.

One of the most ubiquitous tools used in molecular analysis is the micropipette. It is used in distributing very small quantities of substances and loading gels. Because of their expense,

many teachers have not been trained in their use and therefore have difficulty moving beyond this tool to other biotechnology tools such as PCR or gel electrophoresis. Because I teach these skills to 11th and 12th grade IB students, I have a pool of trained students who can mentor others, including teachers.

In most high school classrooms, plants are worked with in traditional ways: investigating germination, transpiration, photosynthesis, and taxonomy. If they are used as model organisms for molecular biology, it is often simply to extract DNA (e.g. strawberries, onions). This unit will use plants, specifically the peanut plant, as the model organism to evaluate the presence/absence of pathogens (TSWV), to extract and amplify DNA, and to run and analyze agarose gels. In so doing, awareness of emerging plant pathogens, the use of plants as model systems in molecular biology, and genetic engineering is increased.

Outline of the Professional Development Day and Activities

7:30 – 8:00 Optional Breakfast/Coffee provided
8:00 – 8:15 Introductions and Orientation to the Day
8:15 – 9:30 Introduction to Micropipetting techniques and practice
9:30 – 9:45 Short Break
9:45 – 11:00 DNA Extraction from peanuts (seeds) and PCR preparation
11:00 - 12:00 Introduction to Emerging Pathogens (PCR ongoing 2hrs. 40 min.)
12:00 - 12:45 Lunch provided
1:00 - 1:30 Intro to TSWV and test strip analysis
1:45 - 2:45 Gel electrophoresis of DNA using Invitrogen E-gels (bufferless electrophoresis) and visualization of bands
2:45 - 3:00 Workshop evaluation

Micropipetting techniques will be introduced using the 1st laboratory exercise found in **The DNA Science** (Miklos, Freyer and Crotty, 2003). In this exercise, teachers (using student mentors) will gain experience using small and large micropipets and colored water, microcentrifuge tubes, and microcentrifuges until they can pipet with less than a 10% error. This is done first because all other lab activities are dependent on proper technique. DNA extraction and preparation for PCR are done next because the PCR will require 2 hours and 40 minutes to run. During that time, a general lecture using powerpoint will be given on emerging pathogens and lunch will be provided. The Tomato Spotted Wilt Virus will also be introduced and the test strip analysis using ELISA – type sticks (much like pregnancy detection kits) conducted. Following PCR, the samples will be placed on 1.2% agarose gels and run using the bufferless invitrogen e-gel bases. Visualization of bands is done with black lights. Finally, the workshop will be evaluated.

Expected Outcomes

It is expected that the 28 middle and high school science teachers will now be able to incorporate the new information on emerging pathogens into their classroom activities and will become more confidant in their approach to using biotechnology in the classroom. An additional benefit is that the 12th grade students should also gain confidence in their skills and experience the personal satisfaction of teaching someone a new skill. Formal feedback will be solicited from these teachers as to how they incorporated the information/techniques of the workshop.

Extension Activity (for a larger grant)

The University of Florida model of a biotechnology equipment locker that can be checked out by different schools within the state of Florida is an excellent model for individual school districts. If interest can be developed, the goal for me would be to work with my district to acquire the expensive tools of biotechnology (micropipets, PCR machines, Invitrogen e-gel bases and

power supplies, micro-plate readers) for loaning within the school district. (Note: all high schools are equipped with vertical and horizontal gel boxes and power sources).

Expertise of the PI

PI is a boarded Ph.D. Medical Geneticist (boarded in 1984; Ph.D. earned in 1976) who has taught lab/lecture courses at West Virginia University, the University of Florida, and Stetson University. For the last 9 years, she has taught Standard Level (SL) and Higher Level (HL) IB Biology as well as Advanced Placement (AP) Biology at Spruce Creek High School in Port Orange, Florida. In those courses, she has incorporated quantitative ELISA, agarose and polyacrilamide gel electrophoresis, restriction enzyme digests, comparative proteiomics using fish and shellfish muscle proteins all using micropipets. Her Higher Level IB Biology pass rate over the last 7 years is 95%, while her SL IB Biology students have averaged an 85% pass rate.

Specific Contributions to Action Proposal

1. The PI will coordinate with the district science consultant, Teresa Northrup, and Staff Development to award inservice points and publicize the opportunity. 2. She will train all student mentors. 3. She will request use of the equipment locker from CPET, in particular the e-gel bases and power cords, PCR machines, and the plant supplies and chemicals necessary to conduct the inservice. 4. She will prepare the classroom for all hands-on studies (placement of pipets and tips; aliquoting all reagents including distilled water; arrangement of vortexes, etc.) 5. She will prepare and give powerpoint presentations introducing the topic of emerging pathogens, the TSWV, micropipeting, PCR and gel electrophoresis. 6. Have the district copy center make copies of all handouts. 7. Put three-ring binders together for all participants. 8. Conduct evaluations 9. Request take-home simulations of Dr. Charles Lawrence of the University of Florida 10. Arrange for breakfast and lunch for all participants.

Literature Cited

Culbreath, A.K., Todd, J.W., and Brown, S.L. 2003 Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 43:53-78.

Gibbs, Paul. College of Veterinary Medicine, University of Florida, Lecture 6/24/09.

Miklos, D.A., Freyer, G.A., Crotty, D.A. 2003 DNA Science: A First Course, 2nd Edition. Cold Spring Harbor Laboratory Press.

Murakami, M., Gallo-Meagher, M., Gorbet, D.W. and Meagher, R.L. 2006. Utilizing immunoassays to determine systemic tomato spotted wilt infection for elucidating field resistance in peanut. Crop Protection. 25: (235-243).

Technical sheet for Agdia ImmunoStrip Tests http://www.agdia.com/cgi bin/catalog.cgi/39300

Budget and Justification

Expected contribution from ICORE Equipment Locker:

8 E-gel bases and power supplies + 15 1.2% agarose gels (1 each per group of 2)
Peanut plants with/without TSWV enough for 14 groups
56 sample bags and 56 Agdia ImmunoStrip Tests (enough for 4 bags/strips per group of 2)
28 micropestles
Seed extraction, preparation, neutralization buffers
Eppendorf and PCR tubes
PCR reaction mix and primers

1 PCR machine with 48 wells

Expenses covered by $200.00

Breakfast and lunch for teachers and student mentors (Up to 40 people: 28 teachers, 9 students, PI and ICORE personnel.)
Micropipet tips, distilled water.
Simulation kits for detecting emerging pathogens (56 ordered: 2 per teacher)

Instead of using the ICORE equipment locker to teach one group of students, it will be used to teach one group of teachers who will be able to incorporate some of this information and techniques in their classrooms. It would be useful to be able to provide these teachers with Dr. Charles Lawrence's microarray simulations.

Update: As of 9/4/09

The Science Consultant, Mrs. Teresa Northrup, has approved the inservice for 6 hours of credit as has the Professional Development Office of The Volusia County Schools. It is scheduled for Friday, October 23, 2009, an ISE day in Volusia County, so that stipends do not have to be given to attendees. It is remarkable that this offering was announced at the district-wide science preplanning meeting the morning of August 19th and on-line registration closed that very same day. I had to open it up to 28 teachers to accommodate 4 more teachers from Spruce Creek High School, the home base for this project, who had waited 12 hours to register and were closed out.

Additionally, I applied for funding from the district and their "Strong Science" initiative (Dr. Chris Colwell, Curriculum Office) and was awarded a grant of $2660.00 to cover the purchase of 7 of the Invitrogen E-Gel systems. As a result, the University of Florida Biotechnology locker with 8 systems will suffice to train 28 teachers.

Lesson Plan

Theme: Emerging Pathogens

Lesson Title: Paying It Forward: Amplifying the Effects of UF HHMI ICORE Emerging Pathogens Summer Institute

Grade Span: Middle-School and High School Teachers (6-12)

Content Emphasis: Science: Biological Sciences including Anatomy and Physiology, pre-IB, IB and AP.

Targeted Benchmarks: To help teachers add enrichment to their curriculum, especially in the areas of evolution (multidrug resistant organisms, genetic recombination of viruses), diversity of living organisms, and biotechnology and its applications.

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Lesson Preparation

Learning goals: What will students be able to do as the result of this lesson?

"Students" should be understood to mean **science teachers.**

1. Students will be able to connect the topic of emerging pathogens to their content areas: e.g. Virus, Bacteria, Protozoans as pathogens of animals and plants; detecting pathogens using biotechnology tools (ELISA, DNA extraction, PCR, gel electrophoresis). Students will learn about the Tomato Spotted Wilt Virus as a model system of emerging pathogens.
2. Students will appreciate the problem of emerging pathogens as it relates to rapid changes in the genetic code of viruses and bacteria and globalalization.
3. Students will be able to competently handle 3 sizes of micropipettes: 2-20 ul, 20-200 ul, and 100-1000 ul with disposable tips, microcentrifuge tubes, microcentrifuge, and vortex mixer. Students will practice with both large and small micropipettes until they can achieve less than a 10% error in pipetting.
4. The "Nevers" associated with the use of digital micropipettes will be emphasized.
5. Students will be able to use a micropestle and extract DNA for PCR analysis from peanuts.
6. Students will mix solutions containing primers, enzymes, buffers to be used in PCR analysis and to load PCR machines.
7. Students will gain experience in loading agarose gels with amplified DNA, running those gels, and evaluating the gets under UV light.
8. Students will be given microarray simulations for detecting emerging pathogens and will understand how to use these simulations in their classrooms.

Estimated time: Please indicate whether this is a stand-alone lesson or a series of lessons.

This lesson is planned for 1 entire school day, from 7:30 am (optional breakfast) until 3:00 pm and includes a 45 minute lunch. Opening remarks will take 15 minutes. Introduction of micropipette lab along with practice and error estimation is slotted for 1 hour and 15 minutes. This will be followed by a small break. DNA extraction and running PCR will follow and take the group until lunch. (PCR will take 2 hours 40 minutes). Following lunch, the lecture on emerging pathogens and introduction to tomato spotted wilt virus will take place for 1 hour, followed by TSWV detection using Agdia immunostrip tests (4 per group of 2: 2 for leaves and 2 for roots). By this time, PCR should be finished and gels can be loaded, run, and analyzed. All that is left is the evaluation of the inservice.

Materials/Resources: Please list any materials or resources relate to this lesson.

From PI laboratory: 1 BioRad MyCycler (thermocycler for PCR); 7 Invitrogen e-bases;
21 micropipettes: 7 each 2-20 ul, 20-200 ul, 100-1000 ul with 3 types of tips. (3 for each pair of students) (Enough for 7 groups of 2 teachers).
2 microcentrifuges for 1.5 ml microcentrifuge tubes and colored water for practice
agarose practice plates with wells.
8 UV lights to read gels
50 Agdia immunostrip test bags; distilled water (4 for each pair of students; enough for 12 ½ groups)
All lab handouts.
Lecture handout. Evaluation instrument.

From ICORE Locker: 1 PCR machine; 8 Invitrogen 3-bases; 15 1.2% safe gels;
24 micropipettes: 8 each 2-20 ul, 20-200 ul, 100-1000 ul with 3 types of tips (blue, yellow, and extended). (3 for each remaining 8 pairs of students)
2 UV lights
58 Agdia immunostrip test bags (4 for each remaining 14 ½ pairs of students)
peanuts with and without TSWV (15 set ups)
peanut plants with and without TSWV (15 set ups)
DNA extraction and PCR kits for 15 student stations

Teacher Preparation: What do you need to do to prepare for this lesson?

Get approval for inservice and 6 credit hours from district and science supervisor (done).
Advertise inservice for October 23rd and register participants (done). Registration is closed.
Prepare 3-ring binder with all handouts and copies of powerpoints for 28 teachers and 9 student mentors.
Order 56 simulations from Dr. Charles Lawrence as take-homes for participants (done).
Prepare 45 minute powerpoint on emerging pathogens using handouts and powerpoints provided by the Emerging Pathogens ICORE seminar.
Prepare laboratory to receive 28 teachers.
Order breakfast and lunch for 28 teachers, 9 student mentors, and 3 instructors.
Train 9 student mentors to help each group of 3 teachers (4 per lab bench total).
Prepare solutions for micropipette training.
Arrange to either prepare tubes for DNA extraction/ PCR or have ICORE bring tubes day of inservice.

Order 7 Invitrogen bases and any gels that ICORE cannot provide.
Obtain evaluation instrument from district.

Lesson Procedure and Evaluation

Introduction: Describe how you will make connections to prior knowledge and experiences and how you will uncover misconceptions.
Begin by asking teachers the following questions (10 minutes):

1. What is an emerging pathogen? List some that you are aware of.
   Draw teachers into definition of: "New, reemerging or drug-resistant infections whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future." Lead teachers into enumerating: HIV Aids, Malaria, Ebola, H1N1 Swine Flu, Bird Flu, Equine encephalitis, St. Louis encephalitis, West Nile virus. TB
2. Why are some pathogens reemerging? What does drug-resistance have to do with it? Use example of H1N1 for elaborating on mutation and recombination of viral genomes within hosts. Use example of TB for multi-drug resistance. Draw out problems in the schools with MRSA. What does globalization have to do with it? Use example of SARS.
3. What is a vector? What is host? Give examples.
4. What are strategies to counter emerging pathogens? Discuss current public health initiatives (vaccination of pregnant women and school age children). Emphasis on handwashing, covering coughs, correct dosing, quarantine. Lead discussion into flu pandemics and public response.

With this as a background, proceed to detailed discussion of AIDS and H1N1 using powerpoints put together with the help of the ICORE powerpoints provided June 2009. Discuss TSWV as provided by Dr.Maria Gallo of the University of Florida.

Exploration: Describe in detail the activity or investigation the students will be engaged in and how you will facilitate the inquiry process to lead to student-developed conclusions.

Everything, with the exception of 1 lecture, will be hands and minds on. Students will be asked to imagine that they are investigating crime scenes, stalking pathogens, detecting genetic clues. As a result, the techniques that they are practicing will take on an additional relevance.
Specific Skills Practiced: 1. Micropipetting, microcentrifuging, % error estimation.
2. DNA extraction 3. DNA amplification 4. Agarose gel loading and running 5. Evaluation of DNA gel banding pattern. 6. Evaluation of peanut plants for evidence of TSWV macroscopically and using monoclonal antibody strip test.

Application: Describe how students will be able to apply what they have learned to other situations.

Although the model system is TSWV in peanuts, students will able to apply what they have learned to the investigation of any emerging pathogen, animal or plant. They will understand the importance of the DNA signature and antibody-antigen response as the bases of diagnosis. They will understand that DNA can be extracted not only from peanuts but from any cell and be amplified to provide enough material for analysis. They will be able to understand the importance of the genetic banding patterns observed in DNA gel electrophoresis.

Assessment: Describe how student knowledge is being assessed at the appropriate cognitive level for the targeted benchmarks.

If the material in the inservice is relevant, teachers will find a way to use it. Finding out how or even if it has been used is the best way to determine if the information/techniques have made an impact. Because teachers all come from the same district, periodic e-mails can be sent soliciting feedback. Quarterly updates will be hoped for. Because everyone is busy, responses to simple questions might elicit a better response than long detailed questionnaires:

a) Have you been able to use the information taken from the inservice to date?
b) Was the powerpoint on emerging pathogens useful?
c) Have you used the simulations yet? Which ones?
d) Do you have plans to use any of the materials in the future?
e) Have you shared any of the labs/information/powerpoints with other teachers?

Other assessments will be done as the inservice proceeds, specifically:
Objectives 1 and 2: Will be assessed by comparing responses given during the first 10 minutes of lecture (assessing background information) with responses of the final 10 minutes (assess capture of key concepts about public health statistics, genetic recombination, and globalization).
Objectives 3 and 4: Will be assessed during micropipette laboratory. The amount of trials necessary to obtain a 0 – 10% error will be recorded and the "nevers" will be monitored by student mentors during the lab to decrease their frequency.
Objectives5, 6 and 7: DNA extraction, PCR, and gel electrophoresis techniques will be assessed by observing final gel product under UV light.
Objective 8: Will be assessed via feedback questionnaire. Utilizing simulations during the school year will constitute a success.

Teacher Self-Reflection: Record your thoughts on the lesson and describe any modifications you would recommend based on the outcomes.

I am very excited to be presenting such a cohesive group of hands-on activities to science teachers and have been amazed at the reception of the proposed inservice. As a result, I am more than a little nervous about expectations. I sincerely want this to be a good use of these teachers' time. They could be planning the 2nd quarter of work, but instead will spend the day with me and my students. I feel like I'm planning a wedding!!! If the lesson goes well, I will be inspired to run a workshop annually. If there is interest, I will gear up to write a proposal establishing a biotechnology lending locker for Volusia County. If expectations are not met, modifications will have to be made. Perhaps the project is too ambitious for 1 day. However, because all teachers are in the same county, it should be easier to trouble shoot these problems and provide additional support to participants.

I am not prepared to lend my school's equipment. But my goal is to expose all teachers, regardless of the area of biology they teach and at the level they teach at, to topics in biology that they will find of interest and could weave into their own classes, imagining projects that their own students could undertake. As all teachers do, I hope to inspire these "students" to learn more, to become excited, and bring some of that excitement into their classroom…even if it is not about emerging pathogens.