Biotechnology

hosa
future health professionals

New for 2022 – 2023
Biomedical Laboratory Science has been split into this Biotechnology event and a Clinical Laboratory Science event. This event is NEW!
A calculator will be provided for the written test for this event.

Event Summary
Biotechnology provides members with the opportunity to gain knowledge and skills required for a laboratory setting using biotechnology. This competitive event consists of two rounds. Round One is a written, multiple-choice test and the top-scoring competitors will advance to Round Two for the skills assessment. This event aims to inspire members to learn more about biotechnology careers.

Sponsorship
This competitive event is sponsored by Bio-Rad Laboratories, Inc.

For resources, helpful videos, and support specifically designed for HOSA and this competitive event, please visit www.biorad.com/hosa

BIO-RAD

Dress Code
Competitors shall wear proper business attire or official HOSA uniform, or attire appropriate to the occupational area, during both rounds. Bonus points will be awarded for proper dress.

Competitors must provide:
- ☐ A photo ID
- ☐ Two #2 pencils (not mechanical) with eraser
- ☐ Ruler (metric, w mm marks)
- ☐ Glasses, safety glasses, face shield or goggles
- ☐ Disposable non-latex gloves
- ☐ Lab Coat (Optional)

General Rules
1. Competitors in this event must be active members of HOSA and in good standing.

2. **Eligible Divisions:** Secondary and Postsecondary/Collegiate divisions are eligible to compete in this

event.

3. Competitors must be familiar with and adhere to the **"**General Rules and Regulations of the HOSA Competitive Events Program (GRR)."
    - Per the GRRs #11 and Appendix H, HOSA members may request accommodation in any competitive event. To learn the definition of an accommodation, please read Appendix H. To request accommodation for the International Leadership Conference, submit the request form here by May 15 at midnight EST.

    - To request accommodation for any regional/state level conferences, please work with your local and state advisor directly. Accommodations must first be done at state in order to be considered for ILC.

4. All competitors shall report to the site of the event at the time designated for each round of competition. At ILC, competitor's photo ID must be presented prior to ALL competition rounds.

Official References

5. All official references, including websites, are used in the development of the written test and skill rating sheets. In addition, some skills have supporting video resources to help competitors prepare for competition per item #14 below.

6. Brown, J. Kirk. *Biotechnology A Laboratory Skills Course*. Bio-Rad. Latest edition.

7. Starr and Taggart. *Biology: The Unity and Diversity of Life AP*. National Geographic Learning Cengage. Latest edition.

8. Biotechnology Careers

Round One Test

9. Test Instructions: The written test will consist of 50 multiple choice items in a maximum of 60 minutes.

10. **Time Remaining Announcements:** There will be NO verbal announcements for time remaining during ILC testing. All ILC testing will be completed in the Testing Center and competitors are responsible for monitoring their own time.

11. **Written Test Plan**
    - Biotechnology industry practices and careers 4%
    - Biotechnology in health……...4%
    - Governmental regulation of biotechnology……...4%
    - Basic laboratory skills 14%
        - PPE
        - Preparing solutions (calculations, use of balance and other equipment)
        - Pipetting
    - Microbiology and cell culture 12%
    - DNA structure and analysis.....14%
    - Bacterial transformation.....10%
    - Polymerase chain reaction (PCR).....14%
    - Protein structure, function, and analysis 14%
    - Immunological applications.....10%

12. The test score from Round One will be used to qualify the competitor for Round Two.

13. At the International Leadership Conference, HOSA will provide basic handheld calculators (no graphing calculators) for addition, subtraction, division, multiplication and square root calculations

14. **Sample Round One Test Questions**

1. What type of bond connects nitrogenous base pairs and holds the two strands of a DNA molecule together? (Bio-Rad pg 114)
   A. Hydrogen
   B. Nitrogenous
   C. Oxygen
   D. Carbon

2. Which discipline of systems biology investigates the full complement of DNA in a cell? (Bio-Rad pg 5)
   A. Microbiomics
   B. Proteomics
   C. Genomics
   D. Metabolomics

3. What was the first bacterium used to commercially to produce genetically engineered human insulin? (Starr pg 240)
   A. Saccharomyces
   B. E. coli
   C. Epstein-Barr
   D. Staphylococci

Round Two Skills

15. Round Two is the performance of a selected skill(s). The Round Two skills approved for this event are:

	Textbook (Bio-Rad)	**Time Allocated**	**Video Resource(s)**
Skill I: Using Micropipets and Transfer Pipets	pp. 50-53 (Part 3)	15 min	Videos 1 and 2
Skill II: Set up Restriction Digestion Reaction	Page 140 (Part 1)	15 min	none
Skill III: DNA Gel Electrophoresis	pp. 140-141(Part 2)	20 min	Video
Skill IV: DNA Gel Interpretation	pp. 136-138, 142	15 min	none
Skill V: Bradford Protein Quantitation Assay	pp. 254-255 (through step 10)	20 min	Video
Skill VI: Bacterial Transformation	pp. 167-171	20 min	Video
Skill VII: Calculation of Transformation Efficiency	pp. 155-156	10 min	none
Skill VIII: Qualitative ELISA	pp. 314-316	20 min	Video

(FOR ALL SKILLS, BODY FLUIDS WILL BE A SIMULATED PRODUCT)

16. The selected skill(s) will be presented to competitors as a written scenario at the beginning of the round. The scenario will be the same for each competitor and will include a challenging component that will require the competitor to apply critical thinking skills. A specific Biotechnology sample scenario can be found HERE.

17. Timing will begin when the scenario is presented to the competitor and will be stopped at the end of the time allowed.

18. The scenario is a secret topic. Competitors MAY NOT discuss or reveal the secret topic until after the event has concluded or will face penalties per the GRRs.

19. Judges will provide information to competitors as directed by the rating sheets. Competitors may ask questions of the judges while performing skills if the questions relate to patient physiology and will be included in the scenario.

Final Scoring

20. The competitor must earn a score of 70% or higher on the combined skill(s) of the event to be recognized as an award winner at the ILC.

21. Final rank is determined by adding the round one test score plus round two skills score. In case of a tie, the highest test score will be used to determine the rank.

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Competitor # _____ Judge's Signature _____

**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill I: Using 20-200 µl and 100-1,000 µl Micropipets and Transfer Pipets (Time: 15 minutes)	**Possible**	**Awarded**
1. Donned proper PPE: glasses/safety glasses/goggles, gloves, and lab coat (optional).	2 0	
2. Labeled 3 microcentrifuge tubes: p200, p1000, and TP. Weighed each tube and recorded its mass. ***Judge verified competitor cleared the balance before weighing each tube.***	4 0	
3. Set a 20-200 µl micropipet to deliver 200 µl. Transferred 200 µl colored water to the tube labeled p200. ***Judge verified competitor (i) selected 20-200 µl micropipet, (ii) set it to 200 µl, and (iii) used a clean pipet tip for the sample.***	4 0	
4. Pipetted another 200 µl of colored water to the tube labeled p200.	2 0	
5. Pipetted 100 µl of colored water to the tube labeled p200. Closed the tube labeled p200 tightly. ***Judge verified competitor (i) selected 20-200 µl micropipet, (ii) set it to 100 µl, and (iii) used a clean pipet tip for the sample.***	4 0	
6. Set a 100-1,000 µl micropipet to deliver 500 µl and transferred 500 µl colored water to the tube labeled p1000. Closed the tube tightly. ***Judge verified competitor (i) selected 100-1,000 µl micropipet, (ii) set it to 500 µl, and (iii) used a clean pipet tip for the sample.***	4 0	
7. Used a transfer pipet to transfer 500 µl colored water to the tube labeled TP. Closed the tube tightly.	4 0	
8. Weighed all three microcentrifuge tubes and recorded mass of each. ***Judge verified competitor (i) cleared the balance before weighing each tube -and- (ii) mass of liquid was similar across all tubes (~0.5 g).***	4 0	
9. Cleaned work area: a. Disposed of pipet tips, microcentrifuge tubes, and transfer pipet in waste receptacle.	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene.	2 0	
TOTAL POINTS - SKILL I **70% Mastery for Skill I = 25.2**	**36**	

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Competitor # _____ Judge's Signature _____

**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill II: Set Up Restriction Digestion Reaction (Time: 15 minutes)	**Possible**	**Awarded**
1. Donned proper PPE: glasses/safety glasses/goggles, gloves, and lab coat (optional).	2 0	
2. Labeled 6 microcentrifuge tubes: CS, S1, S2, S3, S4, S5 and each with own initials.	1 0	
3. Pipetted 10 µl of DNA template into the corresponding microcentrifuge tube. ***Judge verified competitor (i) used a 20 µl micropipet and set it to 10 µl and (ii) used a clean pipet tip for each sample.***	4 0	
4. Pipetted 10 µl of enzyme mix (ENZ) into each PCR tube then mixed by pipetting up and down 2-3 times. ***Judge verified competitor (i) used a 20 µl micropipet and set it to 10 µl and (ii) used a clean pipet tip for each sample.***	4 0	
5. Capped each tube tightly and mixed by flicking each tube with fingers.	2 0	
6. Pulse-spinned tubes in a microcentrifuge to collect all liquid at the bottom of the tube or tapped tubes on table. ***Judge verified competitor balance the tubes in the microcentrifuge or tapped on table.***	4 0	
7. Verbalized one of the two options for incubation: incubating reactions at room temperature overnight -or – incubating reactions at 37ºC for 45 min.	2 0	
8. Cleaned work area: a. Disposed of pipet tips and microcentrifuge tubes in waste receptacle.	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene.	2 0	
TOTAL POINTS - SKILL II **70% Mastery for Skill II = 18.9**	**27**	

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Competitor # _____ Judge's Signature _____

**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill III: DNA Gel Electrophoresis – Digested Samples (Time: 20 minutes)	**Possible**	**Awarded**
1. Donned proper PPE: glasses/safety glasses/goggles, gloves, and lab coat (optional).	2 0	
2. Obtained rack containing 6 reaction samples and DNA size standard. Collected the liquid to the bottom of the tubes by either placing tubes into microcentrifuge or mini centrifuge and pulse-spinning for 5–10 seconds or tapped the tubes gently on the table. ***Judge verified competitor balanced the tubes in the microcentrifuge or tapped them on the table.***	4 0	
3. Pipetted 5 µl of sample loading buffer (SLB) into each tube. Pipetted up and down or flicking the tubes to mix. ***Judge verified competitor (i) selected 2-20 µl micropipet and set it to deliver 5 µl to all 7 tubes and (ii) used a fresh pipet tip for each sample.***	4 0	
4. Placed the precast agarose gel into the electrophoresis chamber. ***Judge verified competitor placed* the wells of the agarose gel near the black (-) electrode or cathode.**	4 0	
5. Filled the electrophoresis chamber with sufficient 1x TAE buffer to cover the gel by approximately 2 mm.	2 0	
6. Loaded 20 µl each sample and 10 µl standard into separate wells into the gel. ***Judge verified competitor (i) selected 20µl micropipet and set it to deliver correct amount, (ii) used a fresh pipet tip for each sample, and (iii) loaded sample into the gel with no gel breakage or sample overflow into nearby wells.***	4 0	
7. Recorded the order of sample loading in laboratory notebook.	2 0	
8. Placed the lid on the electrophoresis chamber.	1 0	
9. Connected the electrical leads to the power supply. ***Judge verified competitor connected red to red and black to black.***	4 0	
10. Turned on the power and ran the gel at 100 V.	2 0	
11. Verbalized that would run for 30 minutes.	2 0	
12. Verbalized completed, then turned off power and removed lid from chamber.	1 0	
13. Cleaned work area: a. Disposed of pipet tips, microcentrifuge tubes, and gel in waste receptacle.	2 0	

Skill III: DNA Gel Electrophoresis – Digested Samples (con't)	Possible	Awarded
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene.	2 0	
TOTAL POINTS - SKILL III **70% Mastery for Skill III = 28**	**40**	

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Competitor # _____ Judge's Signature _____

Skill IV: DNA Gel Interpretation – Digested DNA (Time: 15 minutes)		Possible	Awarded
1.	Using a ruler, measured the distance (in mm) that each of the DNA fragments or bands traveled from the well. Recorded results for each sample and standard in the table provided.	2 0	
2.	Using semilog graph paper provided, plotted the distance versus size for bands 2–6 in the standard.	2 0	
3.	Drew a line of best fit through the points.	4 0	
4.	Used the graph to estimate the fragment size for each band in the crime scene (CS) and suspect samples. Recorded estimates in the table provided.	4 0	
5.	Circled or otherwise delineated which suspect sample matched that of the crime scene (CS).	4 0	
TOTAL POINTS - SKILL IV **70% Mastery for Skill IV = 11.2**		**16**	

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Section # _____ Division: _____ SS _____PS/C
Competitor # _____ Judge's Signature _____

**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill V: Bradford Protein Quantitation Assay (Time: 20 minutes)	**Possible**	**Awarded**
1. Donned proper PPE: glasses/safety glasses/goggles, gloves, & lab coat (optional) .	2 0	
Prepared samples		
2. Labeled two empty microcentrifuge tubes, one **1/50 sample A** and one **1/50 sample B**.	1 0	
3. Pipetted 2 µl of sample A and 98 µl of 1x PBS into the microcentrifuge tube labeled 1/50 sample A. ***Judge verified competitor (i) selected correct micropipet (20 µl micropipet for sample, 20-200 µl micropipet for PBS), (ii) set micropipet to correct volume, and (iii) used a correct pipet tip for each liquid.***	4 0	
4. Mixed well by pipetting, flicking, or vortexing.	2 0	
5. Pipetted 2 µl of sample B and 98 µl of 1x PBS into the microcentrifuge tube labeled 1/50 sample B. ***Judge verified competitor (i) selected correct micropipet (20 µl micropipet for sample, 20-200 µl micropipet for PBS), (ii) set micropipet to correct volume, and (iii) used a correct pipet tip for each liquid.***	4 0	
6. Mixed well by pipetting, flicking, or vortexing.	2 0	
7. Labeled two cuvettes: Sample A and Sample B.	1 0	
8. Pipetted 20 µl of the 1/50 diluted samples into the corresponding cuvettes. ***Judge verified competitor (i) selected correct micropipet (20 µl or 200 µl), (ii) set micropipet to correct volume, and (iii) used a fresh pipet tip for each liquid.***	4 0	
Prepared standards		
9. Labeled eight cuvettes for the protein standards as follows: Blank, 0.125, 0.250, 0.500, 0.750, 1.000, 1.500, 2.000	1 0	
10. Pipetted 20 µl of 1x PBS into the cuvette labeled Blank. ***Judge verified competitor used a 20 µl micropipet to deliver 20 µl.***	4 0	
11. Pipetted 20 µl of each protein standard into the corresponding cuvette. ***Judge verified competitor used a 20 µl micropipet to deliver 20 µl and used a fresh pipet tip for each sample.***	4 0	
Added Bradford reagent		
12. Added 1 ml of the 1x Bradford reagent to all ten cuvettes. ***Judge verified competitor (i) selected correct micropipet (100-1,000 µl), (ii) set micropipet to correct volume, and (iii) used a fresh pipet tip for each sample.***	4 0	
13. Mixed well by pipetting up and down with a 100–1000 µl micropipet (using fresh tip for each sample OR by covering each cuvette with a small piece of Parafilm and inverting the cuvette five times.	2 0	

Skill V: Bradford Protein Quantitation Assay (con't) - Items Evaluated	**Possible**	**Awarded**
14. Incubated cuvettes at room temperature for 5 minutes. (May verbalize time).	1 0	
15. Visually compared the cuvettes containing samples to the cuvettes containing the protein standard to determine the standard that most closely matches the color of each sample; estimated the protein concentration of the samples based on visual comparison. (May verbalize if time is running out).	4 0	
16. Cleaned work area: a. Disposed of pipet tips, microcentrifuge tubes, and cuvettes in waste receptacle.	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene.	2 0	
TOTAL POINTS - SKILL V **70% Mastery for Skill V = 33.6**	**48**	

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**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill VI: Bacterial Transformation (Time: 20 minutes)	**Possible**	**Awarded**
1. Donned proper PPE: glasses/safety glasses/goggles, gloves, & lab coat (optional).	2 0	
Prepared for heat shock		
2. Labeled one microcentrifuge tube +pGLO and another -pGLO.	1 0	
3. Pipetted 250 µl of transformation solution (0.05 M $CaCl_2$) into each tube, placed tubes on ice. ***Judge verified competitor selected 100-1,000 µl micropipet to deliver 250 µl.***	4 0	
4. Selected a sterile plastic inoculation loop. Scraped 2–4 single *E. coli* colonies from the surface of the starter plate.	2 0	
5. Transferred the loop into the +pGLO tube and swirled it in the transformation solution to disperse bacteria. Closed the tube and placed it back on ice. Disposed of loop into biohazard waste receptacle or onto a paper towel for disposal later.	2 0	
6. Selected a second sterile plastic inoculation loop. Scraped 2–4 single *E. coli* colonies from the surface of the starter plate.	2 0	
7. Transferred the loop into the -pGLO tube and swirled it in the transformation solution to disperse bacteria. Disposed of loop into biohazard waste receptacle or onto a paper towel for disposal later.	2 0	
8. Pipetted 10 µl of pGLO plasmid into the +pGLO tube and mixed by pipetting gently up and down. Disposed of pipet tip into biohazard waste receptacle or onto a paper towel for disposal later. ***Judge verified competitor (i) used 20 µl micropipet to deliver 10 µl and (ii) did NOT add plasmid to the -pGLO tube.***	4 0	
9. Placed both tubes back on ice, making sure the tubes were in full contact with the ice.	1 0	
10. Labeled agar plates with initials, date, and "+pGLO" or "-pGLO" as follows: LB/amp +pGLO LB/amp/ara +pGLO LB/amp -pGLO LB -pGLO ***Judge verified competitor labeled the bottoms of the plates and not the lids.***	4 0	
11. Verbalized 10 min incubation had completed.	2 0	

Skill VI: Bacterial Transformation (con't) – Item Evaluated	Possible	Awarded
Performed heat shock		
12. Transferred the +pGLO and -pGLO tubes from the ice into a 42^0 C water bath for exactly 50 seconds making sure the tubes were in full contact with the water. Immediately placed tubes back on ice. ***Judge verified competitor (i) set a timer for 50 sec and (ii) performed heat shock for 50 sec.***	4 0	
13. Verbalized that the tubes remained on ice for 2 minutes.	1 0	
14. Removed the tubes from ice and pipetted 250 µl of LB broth into each tube, using a new pipet tip between samples. Disposed of pipet tips into biohazard waste receptacle or onto a paper towel for disposal later. ***Judge verified competitor (i) used 100-1,000 µl micropipet to deliver 250 µl and (ii) changed the pipet tip between each sample.***	4 0	
15. Removed the tubes from ice and pipetted 250 µl of LB broth into each tube, using a new pipet tip between samples. Disposed of pipet tips into biohazard waste receptacle or onto a paper towel for disposal later. ***Judge verified competitor (i) used 100-1,000 µl micropipet to deliver 250 µl and (ii) changed the pipet tip between each sample.***	4 0	
16. Verbalized the samples incubated at room temperature for 10 min.	1 0	
Plated the bacteria		
17. Mixed the tubes by inverting.	1 0	
18. Pipetted 100 µl of each transformation mixture into the appropriately labeled agar plate using a new pipet tip each time. Disposed of pipet tips into biohazard waste receptacle or onto a paper towel for disposal later. ***Judge verified competitor (i) used 20-200 µl micropipet to deliver 100 µl to each plate, (ii) changed the pipet tip between each sample, and (iii) applied correct sample to correct plate.***	4 0	
19. Used a sterile plastic inoculation loop to spread the bacteria over the entire surface of the plate in all directions. Disposed of loop into biohazard waste receptacle or onto a paper towel for disposal later.	2 0	
20. Repeated for each plate. ***Judge verified competitor used a fresh loop for each plate.***	4 0	
21. Stacked plates together with lids facing downward, agar side facing up. ***Judge verified competitor placed plates with agar side up.***	4 0	
22. Verbalized plates would incubate at 37^0C for 16–24 hours.	2 0	
23. Cleaned work area: a. Disposed of pipet tips, microcentrifuge tubes, and loops into biohazard waste receptacle, cleaned area of any spilled liquid, returned micropipets to rack (if available).	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene.	2 0	
TOTAL POINTS - SKILL VI **70% Mastery for Skill VI = 45.5**	**65**	

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Competitor # _____ Judge's Signature _____

**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill VII: Calculation of Transformation Efficiency (Time: 10 minutes)	**Possible**	**Awarded**
1. Donned proper PPE: safety glasses/goggles, gloves, and lab coat (optional).	2 0	
2. Used a UV pen light to illuminate the Petri dish containing pGLO colonies. Counted the number of green colonies on the plate and recorded that number on the sheet. ***Judge verified competitor recorded the correct number of green colonies.***	4 0	
3. Determined the total amount of DNA used in the transformation from the given data: DNA (µg) = (concentration of DNA (µg/µl) x (volume of DNA in µl) in this experiment, 10 µl of pGLO at a concentration of 0.08 µg/µl was used. ***Judge verified competitor calculated the correct amount of DNA used.***	4 0	
4. Determining the fraction of pGLO plasmid DNA (in the bacteria) that was spread onto the LB/amp/ara plate: 100 µl of cells containing pGLO DNA were plated from a tube containing a total volume of 510 µl. ***Judge verified competitor calculated the correct fraction was calculated.***	4 0	
5. Determined how many micrograms of DNA were spread on the LB/amp/ara plates. ***Judge verified competitor calculated the correct amount.***	4 0	
6. Calculated the transformation efficiency. ***Judge verified the correct transformation efficiency was calculated.***	4 0	
TOTAL POINTS - SKILL VII **70% Mastery for Skill VII = 15.4**	**22**	

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Competitor # _____ Judge's Signature _____

**For all Judge verification steps, full points are only awarded if all components are accurate*

Skill VIII: Qualitative ELISA (Time: 20 minutes)	**Possible**	**Awarded**
1. Donned proper PPE: glasses/safety glasses/goggles, gloves, and lab coat (optional).	2 0	
2. Labeled a 12-well microplate strip: • the first three wells with + for the positive controls. • the next three wells with a – for the negative controls. • the next three wells with an S to indicate the sample.	2 0	
Antigen incubation		
3. Transferred 50 µl of purified antigen (AG) into each well. ***Judge verified competitor used a 20-200 µl micropipet to deliver 50 µl and added AG to all 9 wells.***	4 0	
4. Incubated the samples at room temperature for 2 min.	2 0	
5. Followed wash protocol: a. Tipped each microplate strip upside onto a short stack of paper towels and gently tapped strip a few times to drain the wells while making sure to avoid splashing sample back into wells.	2 0	
b. Discarded the wet paper towels.	2 0	
c. Used a transfer pipet (same transfer pipet can be used) to fill each well with wash buffer, taking care not to spill the buffer into neighboring wells.	2 0	
6. Repeated step #5.	2 0	
Sample incubation (primary antibody)		
7. Transferred 50 µl of the positive control (+) into the three + wells. ***Judge verified competitor used a 20-200 µl micropipet to deliver 50 µl and added + to only the first 3 wells labeled +.***	4 0	
8. Transferred 50 µl of the negative control (-) into the three – wells. ***Judge verified competitor changed to using a new pipet tip, used a 20-200 µl micropipet to deliver 50 µl and added - to only the 3 wells labeled -.***	4 0	
9. Transferred 50 µl of the first sample (S) into the corresponding three wells. ***Judge verified competitor changed to using a new pipet tip, used a 20-200 µl micropipet to deliver 50 µl and added S to only the 3 wells labeled S.***	4 0	
10. Incubated the samples at room temperature for 2 minutes.	1 0	
11. Repeated wash protocol (steps 5 and 6) two times.	1 0	
Enzyme-linked antibody (secondary antibody) incubation		
12. Transferred 50 µl of enzyme-linked antibody (ELA) into each well. ***Judge verified competitor used a 20-200 µl micropipet to deliver 50 µl ELA to all 9 wells.***	4 0	
13. Incubated the samples at room temperature for 2 minutes.	1 0	
14. Repeated wash protocol (steps 5 and 6) three times.	1 0	
Substrate incubation and color development		
15. Transferred 50 µl of enzyme substrate (SUB) into each well. ***Judge verified competitor used a 20-200 µl micropipet to deliver 50 µl ELA to all 9 wells.***	4 0	

Skill VIII: Qualitative ELISA (con't) (Time: 20 minutes)	Possible	Awarded
16. Cleaned work area: a. Disposed of pipet tips, microcentrifuge tubes, transfer pipets, and paper towels into waste receptacle, cleaned area of any spilled liquid, returned micropipets to rack (if available).	2 0	
b. Cleaned work area with surface disinfectant.	2 0	
c. Removed PPE.	2 0	
d. Washed hands or used alcohol-based hand-rub for hand hygiene.	2 0	
17. Observed and reported results. ***Judge verified (+) and S wells were blue, (-) was colorless; competitor confirmed sample was positive.***	4 0	
TOTAL POINTS – SKILL VI **70% Mastery for Skill VI = 37.8**	**54**	