

ARTICLE

Virtual/Remote Labs for Fluorescent Immunocytochemistry or Western Blotting: The Next Best Thing to Being There

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The SARS CoV-2 pandemic forced many college courses to convert to remote instruction almost overnight in the middle of the spring 2020 teaching semester. This article presents two molecular biology labs formerly performed in person by students but converted into virtual labs. The virtual immunocytochemistry experiment teaches the specificity of antibody staining, principles of fluorescent microscopy, diversity of brain cell types and morphologies, and journal article Figure construction skills. The virtual Western blotting experiment teaches sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the specificity of antibody binding, and graph creation and interpretation skills. Both virtual experiments use professionally-produced web-based videos of scientists conducting the lab procedures. Students must answer questions about the techniques and analyze real experimental data generated by past students to take a quiz and write a journal article-style lab report.

At the whole-class level, student quiz and lab report scores from these virtual labs were not statistically different from those from the in-person versions of the same labs from a previous semester, using *t* tests with the Bonferroni correction. On the virtual Western blot quiz, students who did the virtual version actually scored higher than students

who did the in-person version. These results were significant when the 2020 data were analyzed by within-student paired *t* tests for in-person labs done before COVID-19 versus those done virtually after dismissal for all-remote instruction. The students learned the laboratory concepts and data analysis skills just as well virtually as their predecessors had in person. However, the students trained virtually reported that they could not enter the lab and actually do Western blotting or fluorescent immunocytochemistry with their own hands without extensive additional training.

These virtual experiments can be done with data included in the supplemental materials or can easily be adapted for any micrographs or Western blotting images available from previous lab experiments, or in the published literature. When COVID-19 or other public health emergencies necessitate remote instruction and we can't use the best practice of hands-on lab work, virtual labs can be the next best thing to being there.

Key words: virtual laboratory exercise; remote instruction; cerebellum; Purkinje neuron; glia; immunocytochemistry; fluorescence microscopy; SDS-PAGE; Western blot; beta tubulin

In the spring of 2020, the COVID-19 pandemic caused by novel coronavirus SARS-CoV-2 (Harapan, 2020) forced many college campuses worldwide to switch from in-person to remote instruction, sometimes within a matter of days (Ramos, 2020). FUN expanded its website to include resources for teaching neuroscience online (https://www.funfaculty.org/online_neuroscience_teaching). The much-anticipated triennial Faculty for Undergraduate Neuroscience summer workshop at Davidson University had to be cancelled. In its place, FUN organized a virtual summer meeting (VSM) with dual foci on remote instruction and diversity, equity, and inclusion in neuroscience education—proving that even coronavirus cannot stop the FUN.

The Fall 2020 edition of JUNE included several articles describing lab experiments adapted or adaptable for online teaching (Juavinett 2020, Seraphim and Stock 2020, Hanzlick-Burton et al. 2020). This issue presents several more articles on virtual labs originally presented at the FUN VSM. The initial schedule for the FUN VSM lacked ideas for virtual cellular/molecular neuroscience experiments, so the FUN Education Committee solicited additional

presentations in this area. This paper is an extension of a talk given at the VSM to fill that need.

Laboratory skill development is widely recognized as key to preparing undergraduate students for futures in science and medicine (Pinard-Welyczko et al., 2017; Akil 2016; Ramos et al., 2016; Freeman 2014; AAAS Vision and Change 2011; Armbruster 2009; AAAS/HHMI 2009). Active learning and critical thinking facilitate higher-order cognitive strategies (Bloom, 1956; Armbruster, 2009). Undergraduates need to develop the skill of interpreting data (Association of American Medical Colleges-Howard Hughes Medical Institute, 2009; American Association for the Advancement of Science, 2011; Kerchner et al., 2012). Incorporating research and laboratory skills training across the undergraduate curriculum not only produces better-prepared undergraduates, but also makes careers in science and medicine more accessible to more diverse student populations (Buffalari et al., 2020; Chase et al., 2020; Morrison et al. 2020; Hayes 2018; Wiertelak et al., 2018; Hrabowski, 2015; Banger and Brownell, 2014; Steward and Balice-Gordon 2014; Kerchner 2012; Lopatto 2007; Russell et al., 2007). Active learning strategies

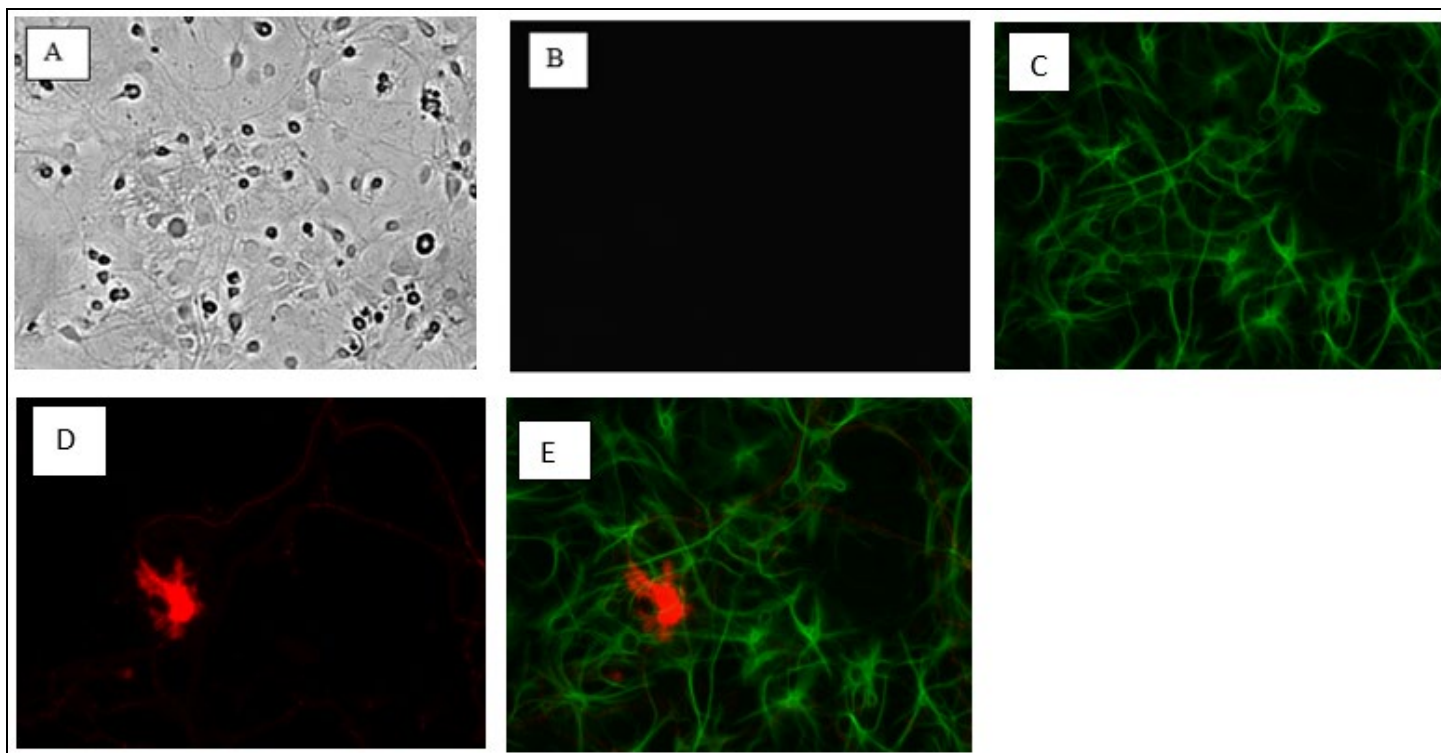


Figure 1. Virtual immunocytochemistry student lab report image panel and legend. Fluorescent micrographs of indirect immunofluorescence of negative control, cerebellum cells, and calbindin in mouse neurons. *A*, micrograph of neurons for positive control. *B*, fluorescent micrograph of negative control containing only the secondary conjugated antibody. *C*, micrograph of neuron filaments stained in cell sample. *D*, micrograph of calbindin stained in cell sample. *E*, superimposing of *Fig. 1C and 1D* to show position of Purkinje cells in cerebellum sample (all images are viewed at 300X magnification using a Nikon TE2000 inverted fluorescence microscope; UV excitation wavelength of 670nm for Cy3 and 493nm for Alexa 488).

including hands-on labs and exercises that require students to grapple with data analysis boost STEM retention, especially among underrepresented minorities (Theobald et al., 2020; Estrada 2018; Hrabowski 2015; Graham et al., 2013; Kanter and Konstantopoulos, 2010; Kuh, 2008).

In the absence of in-person lab experience, as during a pandemic, what can we do to help students develop these vital skills? First, we must model the importance of flexibility, grit, and a growth mindset for our students and ourselves, by transparently demonstrating these qualities and attitudes in our own adjustments to COVID-era teaching (Canning et al., 2019; Duckworth, 2016; Dweck, 2008). Developing or annotating laboratory protocols is still possible and useful when teaching remotely (Ruble and Lom, 2008). Coaching students in the critical analysis and interpretation of research articles and primary literature data can happen remotely, synchronously or asynchronously (Hoskins et al., 2011 Gillen, 2006; Porter, 2005). Zoom breakout rooms allow students to work in groups on case studies or primary literature articles, regardless of where they are physically located (Morrison, unpublished). My students uniformly and deeply appreciated the sense of collaboration, connection, and continuity this approach gave them across the disruptions caused by COVID-19. JUNE also features impactful, ready-made case study activities in almost every issue

(see Rollins, 2020; Ogilvie, 2019; Watson, 2019; Cammack, 2018; Sawyer and Frenzel, 2018; Brielmaier, 2016 for just a few).

This paper describes two cell/molecular biology labs adapted for remote/virtual instruction: one for fluorescent immunocytochemistry of cultured cerebellar neurons, and one for Western blotting of beta tubulin in tissue extracts. Both of these techniques are ubiquitous in the neuroscience literature, including publications on Alzheimer's disease neuroinflammation and cerebellar ataxia models (Zhang et al., 2021; Smets et al., 2015). Assessments of student learning including quizzes and lab reports showed that the students who completed the virtual experiments did as well as—and sometimes better than—those who completed in-person labs. These exercises are usable in courses that lack laboratories, have limited resources, or have to go remote.

MATERIALS AND METHODS

Student Population

These exercises were designed for undergraduate students aged 19-22, enrolled in either a 200-level Introduction to Neuroscience course or a 300-level Immunology course at Lycoming College, a small private liberal arts and sciences college in northcentral Pennsylvania. Course prerequisites included completion of 2 semesters of 100-level introductory biology for both

	maximum possible score	in person 2019			virtual 2020			p value
		average	std. dev.	n	average	std. dev.	n	
Western blot Quiz	20	16.43	3.14	7	19.50	1.18	11	0.0089
Western blot Lab Report	30	25.08	5.17	4	20.54	2.86	8	0.2932
Immunocytochemistry Lab Report	30	24.25	8.49	6	19.66	7.28	4	0.5872

Table 1. Comparison of student assessment scores for in person versus virtual lab experiments. *p* values represent 2-tailed, unpaired *t* tests. Significance level = 0.0083 with Bonferroni correction.

courses, and an additional completion of one semester of 100-level introductory psychology for the Intro Neuroscience course.

Overview of the In-Person Lab Exercises

In-person labs were scheduled for one three-hour period each week, with students working in pairs. The immunocytochemistry experiment occurred over two lab periods, with the first devoted to the immunostaining, and the second devoted to microscopy, image collection, and group discussion of results (see Supplemental Material III). The in-person Western blot experiment occurred over three 3-hour lab periods. Students extracted proteins from meat samples and determined their protein concentrations by Bradford Assay in the first period, loaded and ran SDS-PAGE and set up protein transfer onto nitrocellulose membranes during the second period, and performed the immunoblotting steps and group discussions of results during the third period (see Supplemental Material IV).

Overview of the Virtual Lab Exercises

For both virtual experiments, students received a list of questions about the technique (Supplemental Materials I and II) and were instructed to answer them as they asynchronously viewed professionally-produced videos of scientists from diverse backgrounds conducting the experiments. Then students attended a one-hour synchronous Zoom meeting with the Professor to discuss their answers and any questions they had about the techniques. Their next task was to engage with actual student-generated data from previous semesters and prepare a research journal-style lab report complete with data figures they put together from raw images and data tables. (see Supplemental Materials I, II, and V). They also took a short quiz on each technique the week after the virtual lab day (see Supplemental Materials VI).

Immunocytochemistry of Cultured Cerebellar Cells

Two videos from the Journal of visualized experiments (JoVE) collection were embedded in the Moodle learning management system. Both videos are available as part of either the Education/Advanced Biology/Immunology collection, or the Brain and Behavior collection. These were Immunocytochemistry and Immunohistochemistry: Tissue imaging via light microscopy (13 minutes), and Immunofluorescence Microscope: Immunofluorescent Staining of Paraffin-Embedded Tissue Sections (10

minutes).

Students prepared their lab reports using raw microscope images provided on the course Moodle site, generated by previous students in prior years (Supplemental Material I and V). These images were generated from cultures prepared from postnatal day 0 mice and maintained for 14 days in vitro before paraformaldehyde fixation and immunocytochemical staining. These included bright field, no primary antibody control, rabbit anti-GFAP/goat anti-rabbit Alexa 488, and mouse anti-calbindin D28k/goat anti-rabbit Cy3 images, as well as an overlay image of the GFAP and calbindin stains combined.

Western Blotting of Beta Tubulin in Muscle Tissue: Generation of Raw Data by Previous Students

Students prepared lab reports using data from a previous course's Western blotting experiment (Supplement II). Total protein extracts were prepared from beef, turkey, or pork muscle, and their concentrations determined by comparison with a standard curve of bovine serum albumin using the Bio-Rad Protein Assay Dye Reagent Concentrate (catalog number 5000006). 10 ug of total protein was mixed with 2X Laemmli Sampler Buffer (Bio-Rad catalog number 1610737EDU), loaded into the wells of duplicate 4-15% TGX precast polyacrylamide gels (Bio-Rad catalog number 4561083EDU), and run alongside Kaleidoscope pre-stained molecular weight markers (Bio-Rad catalog number 1610375EDU) in Mini-Protean Tetra Cells using Tris-Glycine/methanol running buffer (Bio-Rad catalog numbers 1658005EDU and 1610744, respectively).

One gel was stained with Bio-Safe Coomassie Stain to reveal all proteins (Bio-Rad catalog number 1610786EDU), while the other gel was transferred onto nitrocellulose membranes (Bio-Rad catalog number 1662807EDU) using Bio-Rad blotting modules (catalog number 1660827EDU). The resulting blots were processed for Western blotting.

Blots were blocked with 1X PBS containing 0.25% Tween20 and 5% nonfat dry milk 2 hours at room temperature with shaking. The next day, blocking solution was replaced with a 1:250 dilution of anti-beta tubulin mouse monoclonal IgG1 (Sigma catalog number T4026) in blocking buffer and incubated for 3 hours at room temperature with shaking. Blots were washed twice with 1X PBS containing 0.025% Tween20, and twice with TBS (150 mM NaCl, 50 mM Tris pH 7.5). Blots received a 1:1000 dilution of goat anti-mouse alkaline phosphatase-conjugated secondary antibody (Sigma catalog number

A3562) in TBS for 45 minutes at room temperature with shaking. Blots were washed twice for 5 minutes with TBS plus Tween, then twice for 5 minutes with TBS without Tween. NBT/BCIP substrate was applied until bands became visible, usually within 5-15 minutes. Substrate was replaced by PBS containing 20mM EDTA to stop the reaction. Gels and blots were photographed.

Western Blotting of Beta Tubulin in Muscle Tissue: Virtual Lab Exercise Used during COVID-19

Three free, publicly-available videos were linked to the Moodle learning management system.

1. The 5-minute Bio-Rad video on the Bradford Assay demonstrates protein concentration determination: <https://youtu.be/vfY3mVOIGBU> (Bio-Rad, 2012). If Bio-Rad's version of this video will not play on your computer, an alternative is: <https://www.youtube.com/watch?v=VewHJNiqom4> (Abnova, 2010).
2. The 8-minute Abcam generic Western blotting video demonstrates sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), protein transfer from gel to membrane, and antibody blotting (but not tissue sample preparation from tissue or cells: <https://www.abcam.com/protocols/general-western-blot-protocol>; abcam, 2020).
3. A 7.5-minute video of an MIT graduate student doing a Western blot demonstrates whole-cell lysate preparation, PAGE, and antibody blotting (but not concentration determination: <https://www.youtube.com/watch?v=u7VwmJw9Gbc> (Meisel, 2013).

The virtual students prepared their lab reports using data from the past years' in-person students described in the section above (Supplemental Material II and V).

Statistical Analysis

Quiz and lab report scores from 2019 in-person labs were compared against those from 2020 in-person labs and those from 2020 virtual labs after dismissal for remote education, using *t* tests with the Bonferroni correction, which reduces the likelihood of type I errors (Sedgwick, 2014). This correction divides the usual alpha (significance) value of 0.05 by the total number of *t* tests in this entire paper (6), yielding a significance cutoff value of 0.0083. At one reviewer's suggestion, the Holm-Bonferroni correction was also calculated. This method ranks the *p* values for multiple comparisons from smallest to largest, and then changes the alpha value for each individual *p* value contingent upon the rank of its *p* value across the multiple comparisons. This reduces the likelihood of type I and type II errors (Holm, 1979).

RESULTS AND DISCUSSION

Learning Goals and General Strategy

The learning goals for the in-person lab exercises included understanding the technical procedures for fluorescent immunocytochemistry and Western blotting, performing these procedures live in the lab, engaging in analysis of real experimental data with all of their imperfections,

converting raw data tables into appropriately-formatted scientific graphs, interpreting graphical data, integration of information from multiple datasets to understand real scientific experiments, and producing journal article-style lab reports. The learning goals for the virtual versions of these lab exercises were the same as for the in-person labs, except that the students could not perform the procedures live in the lab.

Both virtual exercises include the same general stages:

- use online professionally-produced videos
- provide questions to help guide student viewing (these are included in the Supplemental Materials I and II)
- run an open Zoom session to discuss the videos
- use raw student data from past semesters
- have students prepare a research journal-style article as if they had done the experiment themselves, including explaining anomalies in the data (instructions and grading rubric included in Supplemental Materials V)

This workflow can be used to generate additional virtual labs.

Specific Virtual Experiments

The virtual immunocytochemistry lab uses videos from the Journal of Visualized Experiments (JoVE) and microscope images of mouse cerebellar cultures generated by students in previous in-person labs. Students analyze individual and overlaid images of Purkinje neurons (stained with anti-calbindin-D_{28k} and Cy 3) and glial cells (stained with anti-GFAP and Alexa 488). No-primary antibody controls and differential interference-contrast images for the same visual fields are included. Raw images and instructions given to the students are in Supplemental Material I. Figure 1 is an actual data figure generated by a student during the virtual immunocytochemistry activity.

The virtual Western blotting lab uses free online videos from three different sources (see Methods), raw data for Bradford assays to determine protein concentration, an image of a Coomassie blue-stained gel to show total protein, and an image of the completed Western blot from past student work (see Supplemental Material II). This image was chosen deliberately because it is not perfect, and the students have to provide plausible explanations for high background staining and possible degradation of some proteins. They have to create graphs for the Bradford assay results, and for the migration of the protein molecular weight standards versus their size, then use the latter to interpolate the size of putative beta tubulin bands on the Western blot. This exercise is designed to strengthen students' graph creation and analysis skills.

Assessment

The COVID-19 pandemic created an opportunity to compare student performance on in-person versus virtual lab exercises. The virtual labs were created based on previous years' in-person labs, and students in both years took the same short Quiz (see Supplemental Material VI for this quiz and its grading rubric) and wrote a formal journal article-style Lab Report that were graded using the same

	maximum possible score	in-person ELISA			virtual Western or ICC			p value
		average	std. dev.	n	average	std. dev.	n	
ELISA vs. Western blot Quiz	20	16.82	2.95	11	19.50	1.18	11	0.0058*
ELISA vs. Western blot Lab Report	30	21.31	3.25	6	23.03	1.63	6	0.2444
ELISA vs. Immunocytochemistry Lab Report	30	19.02	1.08	3	18.53	8.48	3	0.9288

Table 2. Within-student comparison of assessment scores for in person versus virtual lab experiments. *p* values represent 2-tailed, paired *t* tests. Significance level = 0.0083 with Bonferroni correction. * indicates significant difference.

rubrics across years (see Supplemental Material V for lab report instructions and grading rubric). Table 1 shows Western blot Quiz and Lab Report scores, and Immunocytochemistry Lab Report scores, for spring 2019 (pre-COVID-19, in-person labs) and spring 2020 (post-COVID-19, virtual labs). The post-COVID-19 group did not take the Immunocytochemistry Quiz because the semester was shortened due to a longer spring break to allow the College to pivot to all-remote instruction. The number of students completing each assessment was not constant. The course design allows students to balance their workload across the semester by choosing 10 of 12 Quizzes and 2 of 4 normal Lab Reports to hand in, so not every student completes every assignment.

Using unpaired *t* tests with the Bonferroni correction, the significance cutoff value was 0.0083. No significant differences were found between the in-person and virtual groups for these three assessments (Table 1). However, the *p* value for the Western blot Quiz was 0.0089, very close to significance. Using the Holm-Bonferroni correction, which changes the alpha value contingent upon the rank of *p* values across multiple comparisons (Holt, 1979), the alpha value for this comparison would be 0.01, and this difference would become significant. The virtual students did better on this Quiz than the previous year's in-person students. This may reflect the virtual exercise's shorter and more focused timespan, 1 lab period, compared with 3 consecutive lab weeks for the in-person version of this activity. It might also reflect the general impression that STEM students may earn inflated grades when they take quizzes or exams at home rather than under close proctoring in person (personal communication from numerous colleagues). But I will definitely incorporate the Western blotting videos into pre-lab preparation for future years' in-person Western blotting experiments.

Table 2 shows a within-student analysis for scores from in-person instruction versus virtual instruction from the same spring 2020 semester. All of the students represented in this Table completed a Quiz and a Lab Report on enzyme-linked immunosorbent assays (ELISA) before Lycoming College dismissed all students for remote instruction. They also completed a Western blot Quiz (Supplemental Material VI) and Lab Report (Supplemental Material V), and an immunocytochemistry Lab Report (Supplemental Material V), after dismissal for remote instruction. The students performed better on the Western blot Quiz during remote instruction than during in-person

instruction (Table 2; *p* = 0.0058). It is possible that the inability to proctor the remote Quiz using Moodle, our learning management system, influenced the outcome. To test whether this enhanced Quiz performance represented better overall comprehension of Western blotting, Table 2 also presents data indicating that the virtual students did not perform better on their virtual Western blot Lab Report than they had on their own in-person ELISA Lab Report earlier in the same semester. This may suggest that formal lab reports are a more accurate indication of student understanding than Quizzes when instruction must be virtual. Table 2 also shows no statistically significant difference between the same students' in-person ELISA and virtual immunocytochemistry lab report scores.

Conclusions

This study presents two virtual lab exercises and compares assessment scores for in-person versus virtual labs across years and within-student for the same semester. Statistical analysis found no evidence of significant differences, with two exceptions: students scored better on the virtual Western blot quiz versus the same students' performance on the in-person ELISA quiz (Table 2), and if the Holm-Bonferroni correction is used, two different cohorts of students scored better on the 2020 virtual Western blot quiz versus the 2019 in-person Western blot quiz (Table 1). Student performance on lab reports was not significantly different between in-person and virtual labs. Through the virtual labs, students learn about important lab techniques and engage with raw data, presenting and interpreting them just as they would have had they been able to generate the data themselves. In the process, they develop the same critical thinking skills and higher-order cognitive strategies that practicing neuroscientists use every day.

The general approach taken here can be adapted readily to any other type of experimental data on hand from previous semesters or in the published literature, with the help of online videos demonstrating experimental techniques. I deliberately sought out videos that included a diverse array of scientists. Some of the students even noticed this choice: they spontaneously commented that they appreciated seeing practicing scientists who looked like them. Also, when searching JoVE for neuroscience-relevant video content, it helps to think beyond the Neuroscience collections, to those for Developmental Biology, Genetics, Cell Biology, Immunology, and Microbiology. JoVE's Psychology sub-collections include

Behavior Science, Experimental Psychology, Cognitive Psychology, Developmental Psychology, Neuropsychology, Sensation and Perception, and Social Psychology. The JoVE representatives are quite happy to provide quotations for these sub-collections with prices much lower than the all-access JoVE subscription. And if a JoVE subscription isn't available at your institution, there are plenty of quality experimental procedure videos available free from supply vendors and on YouTube.

While these online/virtual/remote activities can teach some crucial laboratory skills (like data analysis and critical thinking) outside the lab, they likely will not supplant hands-on laboratory experimentation in the long run. Online learning results are greatly enhanced by pairing with hands-on activities (Hanzlick-Burton et al., 2020; DeBoer et al., 2017; Koedinger et al., 2015). So once the COVID-19 pandemic is under better control, I will still use some online/remote/virtual activities as pre-wetlab preparation. Until then—these results indicate that some virtual labs are indeed the next best thing to being in the lab.

Supplementary Materials

Supplemental Material I. Virtual Immunocytochemistry Data Report Results and modified questions

Supplemental Material II. Student instructions and data for virtual Western blot experiment

Supplemental Material III. In-person immunocytochemistry lab manual instructions

Supplemental Material IV. In-person Western blotting lab manual instructions

Supplemental Material V. Instructions and Grading Rubric for Lab Reports

Supplemental Material VI. Western blot Quiz and Answer Key

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