Antibody Validation Roundtable
specificity + sensitivity + reproducibility = validation
Webinar
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Slide 1

Sean Sanders: Hello everyone and a very warm welcome to this Science/AAAS webinar roundtable discussion dealing with Antibody Validation. I'm very pleased that you could join us today. My name is Sean Sanders and I'm editor for custom publishing at Science. A warm welcome to everybody out there that has joined us online.

We're taking a break from our usual format today. Instead of presentations from our panelists, we're going to engage in an hour long roundtable discussion concerning how antibody data reproducibility issues impact academia, industry, and publishing.

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We're fortunate to have with us representatives from all of these spheres, as well as the panelists from an antibody manufacturing company. I think now is a good time to introduce that panel to you.

Dr. Marcia McNutt is a geophysicist by training and editing chief of the Science Family of Journals. She was previously director of the US Geological Survey and prior to that, President and CEO of the Monterey Bay Aquarium Research Institute.

Dr. David Rimm is a professor in the Department of Pathology at the Yale University School of Medicine and director of both Translational Pathology and of Yale Pathology Tissue Services. He was a scientific co-founder of two companies, HistRX, a digital pathology company that was sold in 2012, and the Metamark Genetics, a prognostic determinant company.

Dr. Chris Kerfoot is Co-Founder and President of Mosaic Laboratories. He has extensive expertise in oncology research, companion diagnostics, and product development. Previously, Dr. Kerfoot served as Director of
Product Development at US labs, Director of Pharmaceutical Services at Oncotech, and Director of Molecular Oncology at the IMPATH.

Finally, Dr. Craig Thompson is Vice President of Global Operations and Production at Cell Signaling Technology where he is responsible for manufacturing global distribution and customer support of a broad range of biological reagents including antibodies, antibody conjugates, a variety of assay kits, and companion products. A very warm welcome to all of you and thank you for being with us today.

Before we get started, I'd like to share some information with our online viewers.

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At the top right of your screen, you'll find photos of today's speakers and a "View Bio" link, which you can click on to read more details about their background and research. Underneath the slide viewer is a resources tab where you can find additional information related to today's discussion.

If you're joining us live, we encourage you to submit your questions to the panel at any time by clicking the "Ask a Question" button below the slide window, typing the question into the message box and clicking "OK".

You can also log in to your Facebook, Twitter, or LinkedIn accounts during the webinar to post updates or send tweets about the event. Just click the relevant icon at the top right of the screen. For tweets, you can add the hashtag #scienceswebinar. Finally, thank you to Cell Signaling Technology for sponsoring today's webinar.

I'd like to start the discussion by attempting to define validation or at least provide some clarity about what it might mean in different contexts. Perhaps I could have each of the panelists briefly describe what validation means to you particularly with respect to antibodies and their application.

Dr. Rimm, maybe you'd like to start us off.

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Dr. David Rimm: Thanks, Sean. In fact, thanks first for inviting me to participate since I think antibody validation is an especially important topic. We define antibody validation as it's basically four points that is proving sensitivity
of the antibody, proving specificity of the antibody, that is, it binds to what it's claimed to bind to, proving specificity in the application in which it's going to be used, and then finally proving reproducibility.

Sean Sanders: Excellent! Dr. Kerfoot, would you like to go next?

Dr. Chris Kerfoot: Well, I just like to echo exactly what Dr. Rimm said. We feel the same thing. In terms of the context of this, those various areas apply differently to a diagnostics manufacturer versus a CLIA laboratory, and there are a number of differences there.

Sean Sanders: Great! Thanks for mentioning the laboratory side, the CLIA laboratory side, because that is something that I want to get back to.

Let's jump to Dr. Thompson.

Dr. Craig Thompson: Sure. Thank you, Sean.

[0:05:00]

Certainly, as a manufacturer of antibodies, we strongly support many of the initiatives underway to raise the awareness of the importance of antibody validation, and it's something at CST that we, in fact, take very seriously. We view validation in a couple of important ways, and certainly very consistent with what Dr. Rimm noted.

Importantly for us, when we recommend an antibody for usage or validation, we have to clearly define the recommended usage of the antibody, and that includes what applications, appropriate protocols, and recommended dilutions.

The importance of getting at these include all of the appropriate scientific systems and controls that go along with that, and it's something that we strongly feel is a primary responsibility of the antibody manufacturer to provide that initial validation.

Sean Sanders: Excellent! Dr. McNutt, perhaps you could talk to this from a publisher's perspective and what you're looking for in submitted manuscripts as far as antibody validation is concerned. Dr. McNutt, I think you might still be on mute.

Dr. Marcia McNutt: Sorry. Let me talk about it simply more generally when we look for validation in all contexts, and that is we look for again four points. We look for is there the appropriate accuracy, is there precision,
calibration, and is there repeatability or what is the quality control in the measurement or in the reagents being used?

What we're seeing here in the use of these commercial products is that at one point, the onus was actually on the individual scientists with their lab-grown products to demonstrate this and it is increasingly becoming a part of the commercial manufacturer's responsibility to show that specificity, that it is binding to the desired target, that it is accurate in what it's doing, that it is the quality control so that one batch is doing the exact same thing as the next batch is doing, and that's what we're looking for as a publisher.

Dr. Chris Kerfoot: Sean, let me expand upon that. I know I mentioned different areas and I'm not sure if it's going to come up in detail later, but I mentioned differences between a diagnostics manufacturer, a CLIA lab, and academia.

To expand upon that, for a diagnostics manufacturer, typically with an assay like HercepTest or another class three diagnostic, test results will determine whether a patient is eligible for therapy. You have hundreds of clinical trial samples in concordance with response. You have specificity of at least 3x30 normal tissue types stained with this assay.

You have intraday precision, interpathologist precision, interlaboratory precision, and various robustness tests. This is certainly something that's at a high level, higher than you would see typically for what you would prepare for a manuscript.

From the CLIA laboratories, this is also expanded in that there are now firmer regulations on what a laboratory offering testing services to patients must do in terms of characterizing antibodies with at least, for a basic diagnostic antibody, ten positive and ten negative cancer types.

Right now, I'm just speaking about immunohistochemistry in addition to specificity testing and reproducibility testing. And then beyond that, when you get into something like predictive assays, again, the class three assays, this goes beyond those numbers into 40 cases at a minimum of characterized samples, so there is quite a high level of validations required and I thought it was important to lay that out upfront as we start talking about how researchers will use this guidance.

Sean Sanders: Excellent!

[0:10:05]
Dr. David Rimm: I would like to confirm and add to that; in fact, give you the citation. It's Patrick Fitzgibbons at Principles of Analytic Validation of Immunohistochemical Assays, which is in the archives of Pathology 2014.

That's I think what Chris was referring to in terms of guidelines for validations for clinical labs. That's going to be different than guidelines for validation for research labs, but there is -- for those listeners out there who are interested in the reference, that's the reference for clinical lab validation.

Sean Sanders: Fantastic! Many thanks to that, Dr. Rimm and also Dr. Kerfoot, for that description, and actually, it does bring us nicely to the next point and question that we've received.

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This is around why validation is important. This question says, "... and Dr. Kerfoot described rigorous validation. It sounds like a lot of work and their research resources are limited." So what might be the most important validation experiment that they should look at performing? Maybe Dr. Rimm, you can start us with this one.

Dr. David Rimm: Sure. Thanks! That's a great question because I recently heard that actually from a senior official in an antibody company that they have 18,000 antibodies. So clearly when you're selling 18,000 antibodies, you can't have a really rigorous validation as would be seen in the Fitzgibbons paper, for example, or in our paper, which is a more general antibody validation paper, which is in BioTechniques in 2010.

If I understand the question, really what the questioner is asking is, "What's the minimum that they can do for the least amount of money?" and I would refer them to the same criteria that is sensitivity, specificity, and reproducibility, but in the context in which they're using it.

If they're only going to use this antibody for a Western blot, then they really only have to test it in the Western blot context. If they're going to use it for IHC, I really don't think that they can skimp on any of those areas or they're at risk of producing false results.

Sean Sanders: Great! Dr. Kerfoot, something to add?

Dr. Chris Kerfoot: Absolutely, Dr. Rimm. I think if you were characterizing something for ELISA or for immunohistochemistry, running characterized cell lines or
xenografts that have been characterized by another method, for instance, evaluating Western blot results in those cell lines and then performing immunohistochemistry on formalin-fixed, paraffin-embedded cell lines and comparing the results is very important to evaluate whether the staining in one test method such as immunohistochemistry is matching the amount of protein that you know exists by Western blot.

So I would say that those sorts of tests where you're running characterized samples or samples characterized by an alternative method are some of the most critical portions for academic work.

Sean Sanders: Dr. Thompson, let me come to you just maybe to comment on what Dr. Rimm mentioned about a single company having 18,000 antibodies. What are some of the procedures that you use and what are the minimum validation steps that you take with your antibodies?

Dr. Craig Thompson: Let me comment on in terms of the number of antibodies a company may have. I think it's important to point out, first of all, that a company should only recommend the use of an antibody in a particular application if the appropriate validation has been done on that.

If an antibody is only recommended for western, the end user, if they're going to use it for IHC or some other application, additional validation is certainly necessary, but it is the responsibility of the company selling it to do the appropriate validation for those applications that they are recommending.

But when it comes to the particular validation, the standards in my view should be the same standards applied to any good experimental design, which means using appropriate biological systems, positive and negative controls, and these can be in a variety of forms. We use a wide range of them here at CST, and in general, any one single approach is probably not sufficient.

It's a combination of those things that's critical and that typically includes positive and negative cell lines or tissues, looking at subcellular localization, using specific inhibitors or other types of activators to modulate the expression or localization, various forms to either increase or decrease the level of the target protein such as siRNA or knockout technologies, overexpression.

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It really depends on the biology and it depends on the tools one has available, but it really boils down to what I consider good experimental design. And when somebody is looking at determining which antibody to use or to purchase a particular antibody is to actually look at the data that is provided to you and make a decision as if you were reviewing a publication and decide if in fact that data is sufficient. It's important to make sure that that data is readily available as well.

Sean Sanders: Let me stay with you for a second, Dr. Thompson, to ask a question about the difference in validation between monoclonal and polyclonal antibodies.

Dr. Craig Thompson: Sure. Fundamentally, the initial validation of let's say a new antibody, whether it's monoclonal or polyclonal, is the same. You're really looking at these for the same types of specificity and sensitivity that you would hope for in an antibody.

The difference is more of a technical one. The biggest burden is on the vendor, and that’s the reproducibility. Monoclonals have significant advantages in that the reproducibility is significantly easier to achieve because of the monoclonal nature of it, the ability to manufacture it with greater consistency.

Polyclonals by nature are much more challenging to maintain at the same level of reproducibility from lot to lot due to changes in the animal, changes in the immune response of the same animal, purification protocols, et cetera.

Nonetheless, the quality control that is used should be the same, and that is each lot that's made should be tested against previous lots to ensure reproducibility. And when differences are detected, which can in fact happen often in polyclonals, is that changes in the recommendation to the use of that antibody, whether it is removal of an application or changes in recommended dilution, need to be made clear.

So while it's very critical that every effort be made to maintain the consistency of the performance, it is more challenging in the case of the polyclonal, but the nature of validation is fundamentally the same in both cases.

Sean Sanders: Dr. Rimm, can I come to you for some thoughts on that?

Dr. David Rimm: Yeah, thanks. I think that that's a tricky subject area because we've actually been burned by polyclonal antibodies on multiple occasions,
even purchased from the same vendor. I think you have to -- I don't mean to be cynical, but the vendors usually, their goal is -- their motivation is to increase shareholder value. And so, there's sort of a conflict between their desire to fill an order and their desire to provide high level reproducibility.

What should've happened is this particular vendor should've said, "Well, we don't have that antibody anymore. It's not reproducible from that animal." Instead, we purchased another batch and it turned out to not be reproducible and spent a lot of money and had an assay ultimately fail on the basis of non-reproducibility.

So especially, if you're thinking as a scientist, polyclonal antibodies can be great to do proof of concept, but if you're ever thinking about patient care or clinical assays or use in a context of a companion diagnostic, I think almost you're bound to use a monoclonal.

Dr. Craig Thompson: Yeah, and I would agree. I would certainly agree 100% with Dr. Rimm. When it comes to making choices, I would always go with the monoclonal if you have a choice, and certainly in a case when it comes to dealing with clinical-type applications that's critical.

In regards to certainly companies continuing to sell an antibody, we're not communicating a change in performance. That absolutely is unacceptable. I think there can be consistency in striving to maintain profitability or the bottom line with being open and honest with customers because I think ultimately, getting to the most profitable business is also done by providing the best possible product.

That sometimes includes being honest with the customer about the performance of an antibody, in the case of a polyclonal, as it changes over time, and that is critical. The first step in doing that is to certainly maintain the appropriate level of testing. The second step is appropriate communication about that.

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Sean Sanders: I think we've already drifted into the next topic that I wanted to move to, and that is, what does a researcher need to do to ensure that their experiment conforms with the minimum accepted standards?
This actually comes from a question from an audience-participant. "If the antibody supplier did a rigorous job of validation, does this mean I don't have to do any validation in my lab?" Maybe Dr. Thompson, we'll start with you just to talk a little bit about what your opinion is as far as the product that your company supplies.

Dr. Craig Thompson: Sure. First of all, the initial and primary responsibility for validating an antibody or any product that a company sells is with the vendor itself and that should be whether or not they manufacture that product or simply sell the product. It's ultimately their responsibility.

I would say from the standpoint of an end user, a customer in this case, the first thing they should do is look at the data that's associated with that particular antibody and make sure that if you're interested in using it for a particular application, first of all, that it's recommended for use in that application.

Second of all, the data associated with that is actually presented, and also that appropriate controls are used in that testing to approve that application. That's the first step, is simply reviewing the data that's associated with it and ensuring that it fits your standards of scientific excellence.

In terms of the burden on the end user, there is still -- well, the primary responsibility is on the vendor. There is still some additional responsibility on the end user. As a company, we strive to validate in the most biologically relevant systems with all the appropriate controls. But inevitably, because these are for research use, the ones that we sell at least, the end user is going to be using it in their own system, potentially a variety of species, different types of assays, and it is still incumbent upon the end user to include appropriate controls when they're using an antibody.

That would include positive and negative expression, potential modulation, if that's appropriate. I think the level validation that the end user needs to perform themselves is dependent in part upon how much validation a vendor has done and in part upon how much information in the literature there may be on a particular antibody, but like with anything, I think sound experimental design with appropriate controls that include controls for your antibody are appropriate.

Sean Sanders: Dr. Kerfoot, I'm going to come to you for the industry perspective in just a second, but let me quickly get a brief insight into the academic side from Dr. Rimm.
Dr. David Rimm: Thanks. I actually don't entirely agree with Dr. Thompson. I think if in the best of all worlds, I do, and clearly I don't validate my pipette tip tips for sterility before I use them, nor my cell culture tubes. I just buy them and I use them, but antibodies are a little bit different. They're a little bit more of a high-tech reagent.

In fact, even during the transfer, even though it's a validated reagent -- and Cell Signaling is clearly one of the leading companies in being able to do what I consider rigorous validation -- I still think there's some responsibility on the part of the scientist to validate that it didn't get altered in shipping or some other factor to prove that at least in some way, some of the responsibility for validation is in the lab itself.

As Craig pointed out and I very much agree with, careful experimental design with appropriate controls will often build that validation right in, so it doesn't necessarily need to be extra validation if appropriate controls are used.

Dr. Craig Thompson: I agree with that.

Dr. David Rimm: But I really think that you can use a pipette tip, you can use a culture tube without validating it. You can't really do that with an antibody.

Sean Sanders: Dr. Kerfoot, if you could give us your perspective. I'm going to guess that in your lab, there is always some validation done when a new batch of antibodies comes in.

Dr. Chris Kerfoot: Not just a new lot, but it goes far beyond that. To give you specific cases to point out how critical this is, we're out here in California. Antibodies are often shipped across the country and may end up in a 120-degree warehouse in Arizona.

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If we assumed that even getting the same lot of antibody, a new aliquot of the same lot of antibody, that it was going to perform the same way, we could really impact patient care. So even a new shipment of the same lot of antibody, we have to test that. We have seen new shipments of the same lot, same antibody perform better or worse than prior aliquots of that antibody.
Beyond that, when we talk about monoclonals being preferential to polyclonals, they certainly are, but it is very odd that we have seen differences from lot to lot in monoclonal antibodies as well.

I do not know whether this is contamination at the manufacturing end or something else, but when we were looking at an antibody that was staining epithelia and all of a sudden, with a new lot of antibody, there was staining of nerve, you know right off the bat that you received something with a completely different nature or contamination, so this lot to lot verification is very important.

What we do with every new assay we develop is evaluate a number of different antibodies from manufacturers. We screen them upfront to identify or look at the Western blots the manufacturer has provided online, pick those that produce one clear band, and then optimize an assay to determine whether they're performing consistently with expectations and whether they are working for immunohistochemistry.

Once we have that early data with characterized samples, we'll move them into a full validation using again characterized xenografts, cell lines, or tissue controls. That's tissue controls based on literature, for instance, if you know an antibody should be binding to a marker proliferation in cells.

One might use tonsil and look for the germinal center staining or cells in the germinal center, and then move beyond that, evaluate subcellular localization. Is the antibody performing consistent with expectations and then move into the full validation of specificity, reproducibility, et cetera?

So there is a long process that we undergo. That's for a de novo validation. When we get a new lot in, we have to do lot to lot verification. Even when we get an FDA-approved assay such as the HercepTest, we can't simply run that based on it being FDA approved. We have to do verification before that assay is offered in our laboratory.

Sean Sanders: Dr. McNutt, perhaps I can come to you for a journal perspective considering there have been some fairly high profile cases of retractions over the last few years in a number of different journals with a result that can't be reproduced. I'm sure there are certain cases where antibodies have played a role, so is there something that the journals can do to encourage researchers to do the correct validation on their experiments?

Dr. Marcia McNutt: Absolutely, Sean. I think there's a lot that the journals can do in terms of encouraging best practices. We have constantly been trying to increase
the feasibility of issues like this to raise standards while understanding that this is a community issue that we have to have the funders onboard on one hand because if this is a problem that requires more work on the part of the researcher to do extra validation, then that will require more funding.

If it's something that requires extra training and new approaches to how the research is done, then this is something that needs to have the full cooperation of the leading labs so that this becomes part and parcel of how the next generation of researcher is trained.

It also then has to become part of the journal requirements and expectations of our authors when they submit their work that they can assure the reviewers and the readers that they have followed these best practices. And so, it's a community all working together to make this happen.

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What we're finding on many issues such as this that a lot of this knowledge is right now held within the experience of some of the reading labs and we need to get this more to be written down and codified and understood so that it can be widely disseminated and it's not just because a certain researcher happen to work in the lab of someone that they understand how to do this cross-validation.

And so, their experiments are more trustworthy than someone who came from another lab, which might not have known about these appropriate practices and therefore, their experiments are less trustworthy. This is a whole part of the community helping itself to improve its level of -- raising its standards overall.

Sean Sanders: I'm very pleased that you mentioned community because I actually had a question that I wanted to put to the panel about the role of community especially websites. The ones that I know -- Dr. Rimm, you mentioned in your BioTechniques article ABMiner and antibody pDR2 that I think are fairly well-known, but I know there are a number of others out there.

Dr. Rimm, maybe you could talk to the role that these websites and community organization might play in antibody validation.

Dr. David Rimm: I have some apprehension about that. I think that websites and social media are fun and interesting, but might not be the right place for antibody validation.
As was pointed out just a moment ago by Dr. McNutt, different labs have different sets of standards. And so, while some person might enter that such and such antibody is great validation in their hands, that may be a very low standard compared to if Chris said that an antibody on that same site was validated in his hands. And so, I think that you have to be careful with those things. I'm hopeful that community organizations will build up or that there will be ability to do sort of that function through other standards organizations.

I'm working with one now led by Leonard Freedman called the Global Biological Standard Institute, and they have an effort put forth trying to generate standards for antibody validation that could be broadly or widely published and widely distributed, and even potentially distributed on a web so that there would be best practices or expert best practices agreed upon by multiple experts that would ultimately be distributed in such a way that it could have the broadness of an internet-based antibody reference.

Another similar, the National Institute of Standards or NIST had an initiative. I'm not sure where that's gone, but I think we need standardization that's more of an institute level, if you will, as opposed to a user-based social network level.

Sean Sanders:  Dr. Kerfoot, could you perhaps comment on this, the possibility of some sort of internet database that was from a respected institution?

Dr. Chris Kerfoot: Sure. I think it's an interesting initiative. I'm not aware of anything that I have seen so far that would meet the criteria. What you'd be looking for is those best practices that Dr. Rimm was talking about where it's not just the antibody. It starts with the antibody, but then it's also how the detection system is performing, certain methods that one should avoid.

You could create protocols for each antibody and certainly start with those high visibility targets. An example would be Phospho-Akt or Phospho-ERK from the research setting where there are a number of researchers performing assays, and they're not all done to high quality. Methods using, for instance, Avidin Biotin detection can induce false positive staining in biotin-rich tissues, and so some of this knowledge that is out there should be presented in a website where researchers can look it up and develop best practices.
When we look at literature, oftentimes clients will come to us with a number of different publications and ask us to reproduce the results. Unfortunately, a number of these will have flawed methodology that you're likely causing false positive results, so to eliminate that would really be helpful. I think Dr. Rimm's effort here with the Standards Institute would be very helpful.

Dr. David Rimm: If I can just comment back on that. There's another group that is trying to do some sort of standardization called "Science Exchange" led by Elizabeth Irons, and the idea was that you could exchange for multiple labs and sort of get a multi-institutional validation.

I think that that's a really good concept that's still in its infancy and it is being used by some people as a mechanism to prove validation, is to have it validated in a second lab or a second set of hands. While that's clearly not a gold standard or necessarily best practices, at least it's a step in the right direction.

Sean Sanders: I think this discussion leads us to a good place and to another point that I wanted to bring up, and I'm putting that up for our viewers right now, and that is, what factors play a role in ensuring reproducibility of results and the accurate validation of antibodies?

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A question that came in around this is that -- this viewer says, "Don't you think that sensitivity, specificity, and reproducibility are not entirely the function of the antibody per se, but are strongly affected by the samples used in operative skills and diligence?" and that comes to what we were just talking about.

I think this is especially an issue with sample preparation. Immunohistochemistry, for example, something that I did a lot of during my PhD, was incredibly difficult to standardize because one often didn't know how the samples are being prepared.

Dr. Kerfoot, maybe I'll start with you and you could talk a little bit about this issue.

Dr. Chris Kerfoot: Well, what you just mentioned that it is really the laboratory or the investigator who determines the reproducibility is absolutely true. Looking back at graduate school, there was often for instance in immunohistochemistry, controls were run and one would develop the
assay until the control looked right. From an industry standard, you run the assay the same way every time.

If an incubation step is 30 minutes, it's banded 30 minutes plus or minus 30 seconds on either side, so by doing that, you maximize your reproducibility. And for every assay, we have to determine the percent coefficient of variance and that helps a pharmaceutical company, our client, evaluate whether changes in a biomarker are actually statistically significant or simply the variance of the assay.

In an assay like immunohistochemistry, you have not only the intrinsic assay variability. It could be from the room being a couple degrees warmer on a day or that plus or minus 30 seconds, but you also have differences in the tissue as you cut through it, so you run a control tissue, and that can change as you section through it. All of those things factor into the reproducibility.

When one is doing this at the research level though, oftentimes in early stage research, you're simply running one, two, or ten assays, and as long as you have a control that is identical on all ten of those assay dates. I'm not sure the level that an academic group would have to present to a journal the reproducibility of the assay in their hands. It may be quite an excessive burden that's not needed.

Sean Sanders: Dr. McNutt, maybe you'd like to speak to that point about what a journal might require.

Dr. Marcia McNutt: Yes. Well, what a journal might require in this case -- because we don't have strong and fast guidelines for this -- is primarily what the reviewers are going to be convinced by. I agree with the point that if you have appropriate controls, that may go a long way to showing the phenomenon that the authors are claiming that they have appropriately isolated is indeed there because they are not actually trying to show that they need to have it calibrated exactly in another use case in the same way that it would be necessary in, say, a clinical setting.

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But again, what will convince the reviewers that they have demonstrated to the reviewer satisfaction that they have documented the claims in the paper.

Sean Sanders: Dr. Kerfoot, some comments from you?
Dr. Chris Kerfoot: I agree entirely. I think that if you are running controls and you reference those controls and you indicate that the positive controls were run every day, negative controls were run every day, and the results were similar over the ten different analytical days, if you mention that negative reagent controls were run on every sample and were appropriately negative, I think that gives the reader confidence that this was done in a reproducible manner.

Sean Sanders: Great! Dr. Thompson, you had mentioned controls a little bit earlier. Could you speak about some of the controls that you run and perhaps some that researchers might be able to use in their experiments that would help them have confidence that they've correctly validated their antibodies?

Dr. Craig Thompson: Yeah. I've mentioned some of them before and I think a lot of them, some of the key ones have to do with specificity in regards to showing that your antibody recognizes proteins in the correct samples and doesn't in samples that shouldn't have that.

This can be done by using a variety of positive and negative cell lines or tissues, looking at ways to modulate the expression or localization, using tools that either over-express or lead to decreased expression. These are in fact some of the most critical points to really validating the antibody.

In terms of the more general question about, I think, reproducibility within Science and published results, there are typically many, many variables that go into experiments. One of them is often an antibody, but it's not the only one.

There are questions regarding consistency and protocols and other reagents that go in that, and I think the critical thing there is to maintain the appropriate protocols as others have indicated. I think when it comes to publications, what can often happen is that even when appropriate experimental design and controls are used, oftentimes what is presented for review can be representative data.

I think that's where some of the questions have come up as to the reproducibility, is that what's being submitted in for review to the reviewers and to the editors is representative data. It's that single blot or best case scenario blot, and they haven't shown all the other experiments that were done that were slightly different.

I think those are some of the challenges that are faced within publications, is trying to get at better use of statistical analysis when it's
appropriate and getting a better view of what the level of reproducibility is from experiment to experiment.

Dr. Marcia McNutt: And on that last point, I will say that journals are becoming far less tolerant of cherry-picking experimental results, and that guidelines that are now coming out say things like -- or ask authors whether there were other experiments done that contraindicated the results that are being published and whether the experiment could not be reproduced by the authors, that sort of thing, because this is not considered a best practice to pick out the one out of three that gave the published result and discard the two out of three that did not give the result.

Sean Sanders: Dr. Rimm, could you talk to that as well?

[0:45:02]

Dr. David Rimm: Yeah. I was just going to chime in, but how do we know? I think often reviewers have a single expertise or expertise in a specific area, but then not so much in another area. So sometimes, we see well-refereed papers for one aspect of the -- let's take a high-profile publication where there's a knockout mouse and whole exome sequencing, and then also testing in a clinical cohort.

Well, the testing that's in the clinical cohort might be fine for a referee who's used to refereeing whole exome sequencing, but maybe not so much for someone who referees for New England Journal of Medicine where they're used to being much more critical of biomarkers on cohorts. And so, I think that that's sort of an inherent problem depending on referees to be your gold standard.

I'm not sure that I have a better solution other than the fact, which is what some journals are now doing, is having certain standards or certain criteria that must be met in order to be at a level to be considered for publication.

Dr. Marcia McNutt: Well, I can comment on that. There are a couple of things that we're doing at Science with regard to these issues, which we certainly do take seriously. We realize our growing issues with the scrutiny that we can place on complex research that's being submitted and our desire to assure our readers that we have applied the strictest standards to those papers.

First of all, we are sending our papers out to more reviewers than we have in the past. It was typical that many papers would be seen by two
reviewers, and now with complexity of the teams of authors on the papers, we're finding papers are needing to go to three, four, five reviewers in order to cover the many facets of *Science* that need scrutiny in the papers.

In addition, we've added something called the SBoRE, which is the Statistical Board of Reviewing Editors, which is members of our board of reviewing editors with expertise in statistics simply to look at papers that use statistics either simple statistics or complex statistics to make sure that it has been applied appropriately because there are many evils that can be inflicted upon data analysis simply by inappropriate use of statistics to get incorrect results. We want to make sure that papers are flagged that need extra review by a card-carrying statistician.

**Dr. Chris Kerfoot:** Let me ask you a question. In an era now where data can easily be submitted electronically to support the manuscript and where it could be made available to someone who is reading it later in an online format, for instance, if I was looking at a paper and I wanted to see all the Western blots for an experiment that supported the author's conclusions or all the immunohistochemistry results -- and mind you, the cost of scanning slides or taking images has come down dramatically and we have the ability to make these available online -- is that something that's being looked at?

Because from my standpoint, reading an article, I would have far more confidence if I could see the other 35 tissues that weren't represented in the one figure in the publication.

**Dr. Marcia McNutt:** That's a really good question. You bring up Western blots and other types of data that aren't easily reduced to just a series of bits and bites. We had a long discussion of that in a meeting with the NIH and a number of other leading journals in the biomedical sciences and agreed that this was a goal to be able to archive online for readers and reviewers to see the compendium of work that was done in support of the paper.

*Science* right now does require that all data that was used to support a paper be available to anyone who wants to see it, but right now, to get Western blot data, it's not the easiest thing to do even though we require it of the authors.

**[0:50:16]**

What we're looking into right now is a partnership with a group like Figshare in order to make it easy for authors to simply upload all that
kind of information so that it can be easily shared with anyone who wants to see it.

Sean Sanders: If I could come back to Dr. Thompson, related to all of this is the idea of using reagent ID codes or so-called RRIDs. Dr. Thompson, perhaps you could tell us what these are and how they might impact experimental data that's submitted to journals. Are you there, Dr. Thompson?

Dr. Craig Thompson: Yes, I'm here. It's something that we're aware of it here at CST and we've been discussing it. We're still not sure that the use of unique identifiers is necessary the best solution in part because I think it may be a challenging one to apply across the board.

I think one thing that we can certainly as a scientific community though do a much better job of that will at least I think address the problem is requiring that all antibodies or other reagents used in manuscripts are clearly identified with the correct vendor, catalog number, and complete name of the product, which then includes, in the case of a monoclonal, the actual clone ID as well.

It is still often the case that we see here at CST when we're trying to track, for example, the use of CST antibodies in publications, how often this information is either not included or inconsistently applied. I think in terms of reproducibility, it's often challenging if you can't even identify which antibody was used. It's a very hard place to begin to try and reproduce somebody else's work. I think ensuring that those standards are rigorously adhered to is a good first step.

Sean Sanders: Coming back to you, Dr. McNutt, this question was submitted via email and they ask, "If an editor or scientist discovers that an antibody used in a particular study is incorrect, i.e. for instance, it recognizes the wrong target and that significantly impacts the conclusions of the manuscript, what sort of corrective actions should be taken? Do journals contact authors regarding possible issues with reagents?"

Dr. Marcia McNutt: Absolutely. What we would recommend in a situation like that would be the approach using a technical comment because we've frequently seen in situations like that, authors very much want to be alerted to the fact that there may be a problem with their experiments that may or may not have affected their conclusions.

We've often had situations where authors immediately jump on a situation like that, redo some experiments, and in their response to a technical comment, they will correct the record and either revise their
conclusions, or sometimes if there is a major problem with the original paper, they sometimes could request a retraction of the paper.

So depending on what the outcome is, there's a whole range of possible outcomes of that, but we would very much want to hear from someone who discovers a problem like that because we would definitely alert the authors to the problem.

Dr. David Rimm: That's sometimes a problem. We've on more than one occasion discovered an antibody that didn't validate and thus invalidated two or three papers that were already in the literature.

When we've sent letters to the editor or a note or submitted those papers to various journals, we've often found that the authors of the previous papers become very defensive about their work. Since then, it's not what it appears to be by just simply looking at the journal or the figures or whatever.

[0:55:06]

One particularly egregious infraction was recently where we found a membrane protein shown beautifully in figure one to localize to the nucleus, and we pointed that out to the authors and a letter to the editor. They said, "Well, that was not really what it looked like," as opposed to retracting their work, so I'm not sure how you would recommend handling something like that.

Dr. Marcia McNutt: Well, we have other weapons in our arsenal in cases like that. For example, in a situation where you would write us and in our parlance, it would be a technical comment on the paper, and the authors might respond with their claim that, "Oh no, that could not possibly have affected our result."

If we believe that sounds pretty lame and it's a fairly weak counter to the technical comment, the editors can publish an editorial expression of concern about the results of the paper, which goes into the published literature, which basically says that Science no longer has any confidence in the results of that paper.

So what that says is that while the authors have not agreed to retract it, the journal no longer supports the findings in it. That often triggers a reaction from the authors just to have that editorial expression of concern, and that is noted on Retraction Watch that that has been issued.
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Sean Sanders: Great! We have a couple of minutes left in the webinar and I'd like to just come to a couple of quick audience questions that have come in that are sort of peripheral to what we've been discussing.

The first one, and perhaps I'll put this to Dr. Thompson, "To validate the specificity of an antibody, would you think it important an antibody targeting an intracellular protein should have immunohistochemical study showing its intracellular location? This is especially important for antibodies raised in short peptide fragments," this viewer says. What are your thoughts on that?

Dr. Craig Thompson: I think in terms of showing its localization in a cell by immunohistochemistry, it would really depend on in terms of the validation and recommended applications. For example, if Western blot only were the application that it was recommended for, having appropriate localization in a staining application, it wouldn't be necessary. There are a number of reasons why it may not stain appropriately, but it may work perfectly fine in Western blotting.

Of course, if the desired application is something like IHC or immunofluorescence, whether it's on cells or on tissues, of course having the appropriate localization along with other types of supporting data is absolutely critical, but again, it really boils down to what the recommended application is. If Western blot is the recommended application, to correct staining in another application is not necessary.

Sean Sanders: Just one last question very briefly on the last minute that we have to Dr. Rimm and Dr. Kerfoot, this viewer asks, "If a research is planning on purchasing an antibody for a new experiment, what should they be looking for from a supplier as far as validation information is concerned?"

Maybe you could just give a brief answer. What would you look for when looking at an antibody to purchase? Dr. Rimm?

Dr. David Rimm: I would first look for what they do for validation. Many of the spec sheets for the antibodies -- and in fact, if you go to Cell Signaling's website, you can find lots of validation just by clicking through. And so, it's pretty easy to decide if it's been appropriately validated for some things.

Other antibody websites are much less transparent and harder to see, but as a general rule in my lab, what we do is we start with companies
that we have a good track record and then we check for the validation that they're willing to put up online, including all those factors, sensitivity, specificity, reproducibility, and specificity in the assay in which we intend to use them.

[1:00:10]

Dr. Chris Kerfoot: Absolutely, and you start with the Western blot. Pay attention to make sure that the band is at the right level. It's surprising how many Western blot results from different manufacturers have the wrong kilodalton weight.

Dr. David Rimm: But you have to be careful with that, Chris. We've actually validated a number of antibodies that don't work on Western that are beautiful in IHC. So the fact that it fails on a Western doesn't necessarily eliminate it if it makes it through the siRNA knockdown and overexpression series in cell lines. Even though it doesn't work on a Western blot, it may still be a valuable antibody.

Dr. Chris Kerfoot: Absolutely. Good point.

Sean Sanders: Fantastic! Well, unfortunately we are out of time, so we're going to have to end there. On behalf of myself and our online audience, I want to thank our panelists very much for being with us on the line today and for providing such engaging and enlightening discussion, Dr. Marcia McNutt from Science, Dr. David Rimm from Yale University School of Medicine, Dr. Chris Kerfoot from Mosaic Laboratories, and Dr. Craig Thompson from Cell Signaling Technology.

Please go to the URL at the bottom of the slide that should be coming up in your slide viewer.

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Again, thank you very much to our panel and to Cell Signaling Technology for their kind sponsorship of today's educational seminar. Goodbye.

[1:02:01] End of Audio