IPQ INTERNATIONAL PHARMACEUTICAL QUALITY
Inside the Global Regulatory Dialogue

CBER'S ROBIN LEVIS ON THE PROGRESS IN ALTERNATIVE VACCINE ASSAYS

At the April 2019 Global Health Fund (GHF) workshop on replacing animal-based release testing for vaccines, CBER Division of Viral Products Deputy Director Robin Levis reviewed the "smorgasbord" of collaborative activities with which the agency has been engaged. She discussed: ● removal of the general safety test ● moving from monkey to rat-based mumps virus vaccine safety tests ● conventional adventitious agent testing vs. high-throughput sequencing ● changes in acellular pertussis vaccine safety testing, and ● the in vitro rabies potency assay. CLICK HERE for the slides accompanying Levis' talk. Formatting changes and other minor edits have been made by IPQ for clarity and readability. The normal disclaimer that the presentation represents the views of the speaker and not necessarily that of his/her organization is not included.

I was originally going to spend my allotted time talking about the efforts on alternative assay development for rabies virus vaccines because it really is an area that has been very rich with activity over many decades. It makes for a very nice case study of everything that is good and bad about trying to do these activities. But then in thinking about an FDA perspective, there are actually a lot of activities going at FDA with regards to replacement assays.

So I have kind of changed my talk a little bit, and before I go any further I am going to do my acknowledgements because I think I am going to run out of time at the end. These are just folks at the FDA and collaborators outside of the FDA that have been involved in some of the work that I am going to talk about, both from a regulatory point of view and from a research point of view – a science point of view ***[see slide #3].***

And now to get back on message: The take-home message that I want to bring to everyone today is that **research** is really critical for all of this. I would think everyone in this room would already know that. But the development of new technologies and new ways to produce vaccines really improves our ability to make available state-of-the-art-vaccines. That is important whether you are an academic researcher, whether you are an industry person, or whether you are a regulator.

E**volution** is also really important. We need to create and utilize pathways that allow for the introduction of new technologies while retaining the safety and purity of vaccines.

Next, the best efforts are **collaborative efforts**. If I don't say it for every single project that I am going to talk about, it is not just that it has been an FDA effort – it has been truly a global effort to move the development of alternate assays forward. That to me is really important.

And now speaking personally and from an FDA perspective – **science** does drive our regulatory decision making. I was really happy to see that Ivana [Knezevic] and her slide also said the same thing with respect to efforts and work that is going on at WHO.

Today I am going to go through a smorgasbord of activities that have gone on at the FDA with respect to the 3R activities. The ones highlighted in red I am going to give you a couple of slides and examples of. If I can just go through the list quickly:

- With respect to the **reduction** of animal testing, the FDA was one of the global leaders in getting rid of the general safety test [GST].

● Secondly, with respect to the **refinement** of animal testing, we had a really beautiful set of laboratory experiments on mumps vaccines and the replacement of the monkey neurovirulence test with a rat neurovirulence test. So it is not a complete removal of the animal test, but it is clearly a very important refinement thereof.

● And then with respect to **replacement** activities, I am going to highlight briefly some of the work on adventitious agent testing and the replacement of the animal assays with high-throughput sequencing as a potential. Also to speak to one thing that Ivana said, 'that it is time for regulators to get involved in this conversation.' Well we certainly have been involved with this conversation for some time, and I will show some slides with regards to that.

● Next with regards to the DTaP vaccine, there was a large global effort to replace or remove the lethal histamine sensitization assay in mice and replace it with an *in vitro* CHO cell-based assay. That was work that was done at FDA, Health Canada, and other global laboratories.

● What I am not going to talk about is a change in the inactivated polio vaccine, although after Ray [Prasad's] introduction I probably should have.

● There has been work with the replacement of the rat immunogenicity test with an antigen ELISA. With regards to the Hepatitis B vaccines and the HPV vaccines and other vaccines as well, we very successfully have reviewed data and replaced immunogenicity tests in mice with the ELISA assay. I will do this as an introduction of Bob Citron's talk, which will come up and he will do much better service to the HPV vaccine than I would.

● Then finally, with the time that I have left – like I said I could talk hours about rabies vaccine work – I am going to try to just spend a very short time to go over the global efforts on replacing the lethal challenge in mice with an antigen ELISA.

Removal of the General Safety Test

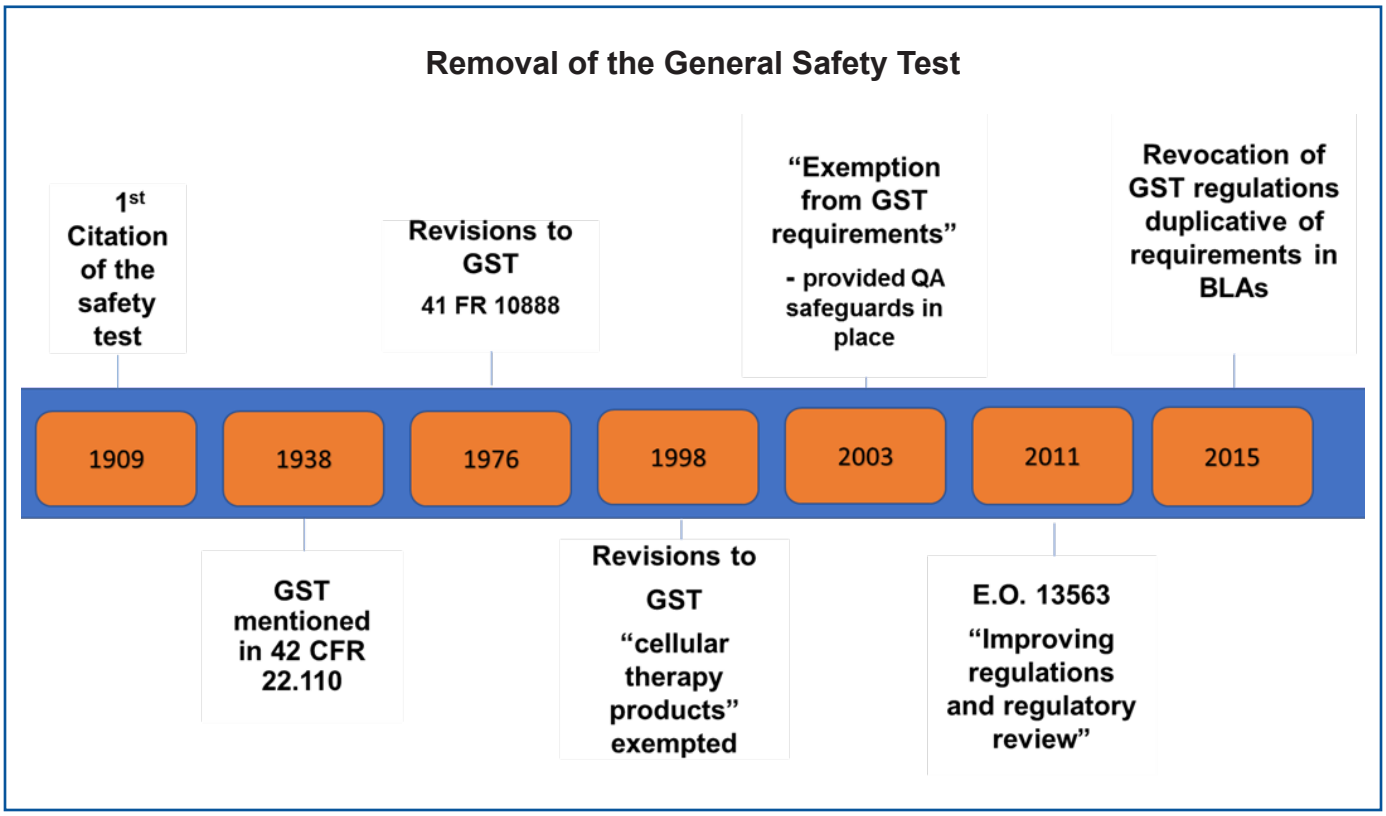
To start off with, the removal of the general safety test: This is a timeline for the general safety test in different major activities, but if you look back over a hundred years ago, was there a need for a test like the general safety test? When there was phenol in biological products and vaccines, when there were other solvents, when there were additives that were of uncertain safety or characteristics, there probably was a need to have this type of assay in place.

But with the advent of GMPs and consistency of manufacturing there really was no longer a need to do this test, which did not tell us very much anyway and was not a very scientifically sound test. So I would highlight that starting in 2003 we were able to allow or grant exemptions from performing the general safety test based on some changes in the guidelines.

At that time then, we received numerous supplements that were submitted from industry to remove the use of the general safety test from finished products, and we granted those.

However, in 2015 we were actually able to revoke the general safety test and remove it from the CFR. That was a huge administrative, legal, and regulatory effort. But once it was done, now we no longer require the general safety test at any point of product development or through the licensure pathway.

Those products that were licensed with the general safety test in their license, the industry partners and the sponsors need to submit a request to us to remove the general safety test from their license. Provide the good data, a good safety profile, and good manufacturing data profile, and we allow that removal.



Monkey to Rat-based Mumps Virus Vaccine Safety Test

Next, I am going to speak about the mumps virus vaccine safety test. The mumps virus is a highly neurotropic virus, and in most human cases there is CNS [central nervous system] involvement from mumps infection. What has been shown historically is that many vaccines are also associated with aseptic meningitis, and those strains are listed here: [Urabe AM9, Leningrad-Zagreb, Lenenigrad-3, Sofia-6, Hoshino, Torii]. What has happened when those vaccines end up on the market is then the adverse events are noted, and the vaccines are withdrawn from the market.

A safety test for the mumps vaccine that previously assessed neurovirulence was a monkey neurovirulence test. Two important things: This test was based on the test that was originally designed for the polio virus neurovirulence safety, which we know had proven very important and significant with respect to polio virus. Interestingly enough – and I just learned this in preparing this talk – this test was translated to be used for mumps, but it was never validated for use with mumps, and the presumption was that it would behave similarly.

That being said, it was of questionable predictive value. Vaccines which caused meningitis in humans passed the monkey neurovirulence safety test. If we look at this slide, it just shows what the monkey neurovirulence safety test is after immunization or brain sections are taken. In the blue stains parts, the lesions scores are scored 1 to 4-plus based on the response to the vaccine. If you look, this is again the monkey neurovirulence test, there is really no discrimination in monkeys among the mumps virus strains, which we know based on human use have a very neurovirulent potential ***[see slide #10]***.

So what is the new test? A rat-based assay was developed in the perinatal rats, which are exquisitely sensitive to infection by mumps. So for this test, you can see vaccine virus versus wild-type virus and what the morphology of pathology in the rat brain is, and there are two other slides.

If we do a test comparison, this is actually the rat neurovirulence test and vaccine A and vaccine B – one licensed in the US and one in Europe – and generally used. The next two, the Urabe vaccine is the one that was shown to have high reactivity and was removed from the market, and then some wild-type isolates.

If we compare that with the original monkey neurovirulence test, you can really see the lack of discriminatory power within the monkey neurovirulent test ***[see slide #12]***.

With respect to the implementation of the rat-based test, it was validated in a multicenter study in partnership with the NIBSC [National Institute for Biological Standards and Control]. It is now used by vaccine manufacturers. It eliminates the needs to use monkeys, and it has been the foundation of development of new nonanimal alternatives to neurovirulence testing. In addition, this test has been used to look at attenuation phenotypes for new vaccines under development.

Conventional Adventitious Agent Testing Versus High-Throughput Sequencing

My next topic is going to be moving onto conventional adventitious testing versus high-throughput sequencing. In the first set of bullets here are the conventional test modalities that are used for adventitious agent testing. The four highlighted in blue are those that are the animal-based assays.

As well as using a large number of assays, these tests take a very long time, up to 42 days in the guinea pigs with respect to the observations post-inoculation. So, everyone is talking about replacement testing and one is the high throughput sequencing technologies, which are an advanced nucleic-acid-based technology that really has the ability to detect known and unknown viruses without prior sequence knowledge.

Conventional Adventitious Agents Testing vs. High Throughput Sequencing

► **Conventional testing**

- **At least 3 cell types, 28 day observation (subculture)**
- **Transmission Electron Microscopy**
- **Biochemical and infectivity tests for retroviruses**
- **PCR for specific human, bovine, and porcine viruses**
- **Embryonated chicken eggs, 2 routes, 18 days (blind passage)**
- **Adult mice, 21 day observation**
- **Suckling mice, 28 day observation (blind passage)**
- **Guinea pigs, 42 day observation**

► **Replacement testing**

- **High Throughput Sequencing (HTS) is an advanced nucleic acid based technology that has the ability to detect known and unknown viruses, without prior sequence knowledge.**

Examples of that are the paper on the finding of the porcine circovirus in a couple of vaccines, and also work under the FDA where there was the finding of a rhabdovirus in a cell line used to produce baculovirus base.

How would we use high-throughput sequencing for virus detection in biologics? Well it could be used as a replacement or an alternative assay to the *in vivo* and PCR assays.

[Compared to] the *in vivo* adventitious virus assay, the HTS can provide defined sensitivity and breadth of virus detection. HTS can have a similar or greater sensitivity than the PCR assays but a broader virus detection in a single assay. Industry and regulators are not left then with trying to come up with this complex list of what is the appropriate PCR assay to do. You can literally do one-stop-shopping with your HTS.

In addition, it can serve as a supplementary assay to the *in vitro* assays, which are non-animal based assays. But with regards to cell substrate characterization, there are concerns for occult or novel viruses that won't grow in a specific substrate but may still be there. The high throughput sequencing can detect those.

One of the issues we have with regards to *in vitro* adventitious agents testing if you have a live virus vaccine is having to neutralize the virus prior to inoculating cells for assay. That can oftentimes affect the readout of an *in vitro* assay, whereas the high-throughput sequencing would preclude the need for neutralization.

One of the critical things about using the high-throughput sequencing is what do we do if we find a positive result. And that is true for any nucleic-acid-based test. But given the great depth of data that we all obtain using the high-throughput sequencing, it is going to be necessary to define a path forward with respect to how to control positive hits.

Advanced Virus Detection Technologies Interest Group [AVDTIG]

So I am going to put a plug in here for the Advanced Virus Detection Technologies Interest Group, which is a very large, global group of regulators, industry folks, and academicians at universities who have come together and work on establishing good methodologies for the use of high-throughput sequencing in industry.

I just put this publication here if anyone is interested in following up. This is an open access group, anybody can join and go to the meetings. Basically the group is divided into four subgroups, which then each work on a specific aspect of high-throughput sequencing and moving it forward for use as part of the adventitious agent testing ***[see slide #16]***.

I think us embracing the technology of the high-throughput sequencing is a really good example where we are evolving into using modern technologies to address old questions, both from an industry and regulatory point of view. What is important is identifying the challenges and filling in our knowledge gaps. Both industry and regulatory roles in moving these efforts forward are really important in terms of databases, bioinformatics analysis. What does a regulator need to see to ensure and review this data appropriately?

There have been numerous early discussions between industry and regulatory authorities. These can help identify and address scientific challenges because the technology is still evolving. It is really critical to have assay and bioinformatic standardization and validation of these assays. That may look different from each industry or partner or for each product type or whether it is a cell type or a cell substrate versus a virus seed.

Those are all discussion going on. Really what are the critical regulatory issues? Are we going to ask for the raw data and reevaluate the whole package? I don't think that is necessary, but maybe it is in some instances. So there are a lot of issues that we are working through as regulators in terms of how we interface with the data and use it best for the quality control.

Again, I would just highlight this has been a really collaborative effort, both amongst stakeholders but also globally in terms of moving this forward.

Changes in Acellular Pertussis Vaccine Safety Testing

I am going to quickly introduce the safety testing changes in the testing for the acellular pertussis vaccine. Again, this was a large collaborative group. With this I am just going to the highlight work at the FDA in [OVRR Principal Investigator] Drusilla Burns' lab.

The original animal test was a murine histamine sensitization test, which is a lethal challenge test in mice. It is a pretty nasty death of these poor mice. It was based on sensitization of the mice to histamine by the active pertussis toxin.

The replacement test on the other hand, which has now gone all the way through EDQM, and the monographs have been changed to remove the histamine sensitization test. The replacement test is an *in vitro* test in CHO cells. It is based on a distinct morphology that the cells take on in the presence of pertussis toxin. This is a slide to just highlight the normal pathology versus the clustered pathology in the presence of the PT-spiked vaccine.

So this is a really nice story of *fait accompli* of people recognizing a bad test and then work done to move forward to replace it.

In Vitro Rabies Potency Assay

Now I am going to just spend my remaining time on the rabies *in vitro* potency assay. Just in general, there has been an ongoing global effort by vaccine manufactures and control authorities to establish alternative rabies virus vaccine potency tests using current state-of-the-art technologies. That bullet point has been used for 50-plus years. I am not kidding.

These efforts are driven by a scientific need for a better assay, and like I said, those discussions have been going on for 50-plus years. But within the last couple of decades also this response has been to an awareness of animal welfare.

We have already had the introductions from Ivana on what the test is, but I will go over that briefly.

This is a modern-day bullet point: These efforts have really finally led to a well-defined strategy for test replacement to the extent that the EDQM biological standardization program has been willing to take this on as one of their programs and are now planning a collaborative study. So the results could lead to a revision of the European Pharmacopeia, which is what EDQM's bases are, but for the rest of the world it could then ultimately lead to changes in the product license.

Just a very brief history of the NIH potency test: It was developed and published in the 'laboratory techniques and rabies' WHO series back in 1966. And it was adopted for the use as a potency assay for the very first licensed rabies vaccines. And remember, the very first licensed rabies vaccines were neurotissue-derived and administered interperitoneally.

But the test basically requires immunization of groups of mice, 16-20 mice per group, with dilutions of test and reference vaccines on day zero and seven, followed by intercranial challenge with live virus challenge on day 14. And then the LD 50 or the ED 50, depending on which side of the cup you look at, is calculated and the potency is determined relative to the standard at day 28.

It was immediately recognized as a problematic assay. Why is it problematic? Well from an animal welfare point of view, it uses approximately 300 mice per test, and the original protocol was an observation for death. Within the last ten years a humane endpoint has been put in place for the death signal, so now there is an observation for signs of rabies disease.

As it has already been said, this is another one of these biological assays where there is a really high degree of variability – 25 to 400%. And to bring up a point that Ray [Prasad] brought up, the validity criteria are really difficult to meet for this test. So the number of retests that are done is tremendous. So in the US, we have a pass on potency as the geometric means of two valid tests, so that can take a very, very long time.

Replacing the Existing Test

So can we replace this test? Well we are hoping. We have successfully approved the replacement of several animal-based immunogenicity assays with ELISA-based assays. Those replacements were based on: ● neutralizing epitopes being well defined or ● antibody used in the assay bind to the critical conformational epitopes that can be measured, and ● the clear correlations between the amount of antigen required to induce the immune response in animals versus the amount of antigen measured using alternative assays versus immune response in humans. [These studies are most successfully conducted as part of clinical development.]

Can we do it with rabies? I am just going to go quickly through these slides, and I am going to only highlight in this one that in 1984 there was a huge collaborative study to try to replace the NIH test with an SRD test that had been developed in France. There were 14 labs involved, seven countries, it was a huge WHO collaborative study, and it failed miserably because one of the predefined endpoints was it had to correlate with the NIH tests.

If you have a test that is 25-400%, and you have a test that is 20% – I am going to let Tim talk about this in his talk – there was no way there was ever going to be a direct correlation between these two assays. It was just a beautiful piece of work when you look at the data. But based on this prespecified condition of correlation, they failed.

Global Efforts to Develop an Alternate Assay

- **1966 – NIH potency test defined**
- **1984 – Collaborative Study: SRD/NIH potency test –14 labs, 7 countries**
- **1985 – Workshop on NIH potency test –Geneva, Switzerland**
- **1991 – Workshop on rabies vaccine potency testing –Malzeville-Nancy, France**
- **1992 – Collaborative study: in vitroassays/NIH potency test –4 labs, 49 lots of vaccine**
- **1992 – Collaborative study: calibration of the 5thIS**
    - Performed using all available assays –SRD, ABT, EIA, NIH
    - First reference defined using multiple test modalities

Workshop Discussions

Then I am going to hop forward to a workshop we held at the FDA to reinitiate discussions. That was in 2000, and that kind of got the ball rolling again with respect to industry partners and regulators being interested in thinking about replacing this test.

And then it was not for another decade that there was a workshop in Ames, Iowa that was a really good workshop. It had both human and veterinary folks there, and people were very passionate about getting these replacement tests back on the books.

What this coincided with was a law being passed in Europe, which outlawed all use of animals in products for human use. So now all of a sudden, industry and other regulators were also more interested in joining these conversations.

But two important points from that meeting were that the NIH tests should be replaced as soon as possible, and that the current *in vivo* tests should not be used for correlation.

Global Efforts to Develop an Alternate Assay

- **2000 – Workshop to re-initiate discussion on alternate test development –Bethesda, Maryland**
- **2005 – *Creation of the EPAA –European Partnership for Alternative Approaches to Animal Testing***
- **2010 – Workshop on the consistency control of vaccines –Strasbourg, France**
  - Rabies vaccines were one of the topic vaccines
- **2011 – Workshop on alternate rabies virus vaccine potency test development – Ames, Iowa**
  - NIH test should be replaced as soon as possible
  - Current in vivo test should not be used for correlation

Along with those efforts there was a workshop held in France in 2012 that was sponsored by EPAA and ECVAM. It brought together all of the people who have been talking about this for years – some of them with canes, I am not kidding – to kind of reinitiate the conversation and define what really needed to get done.

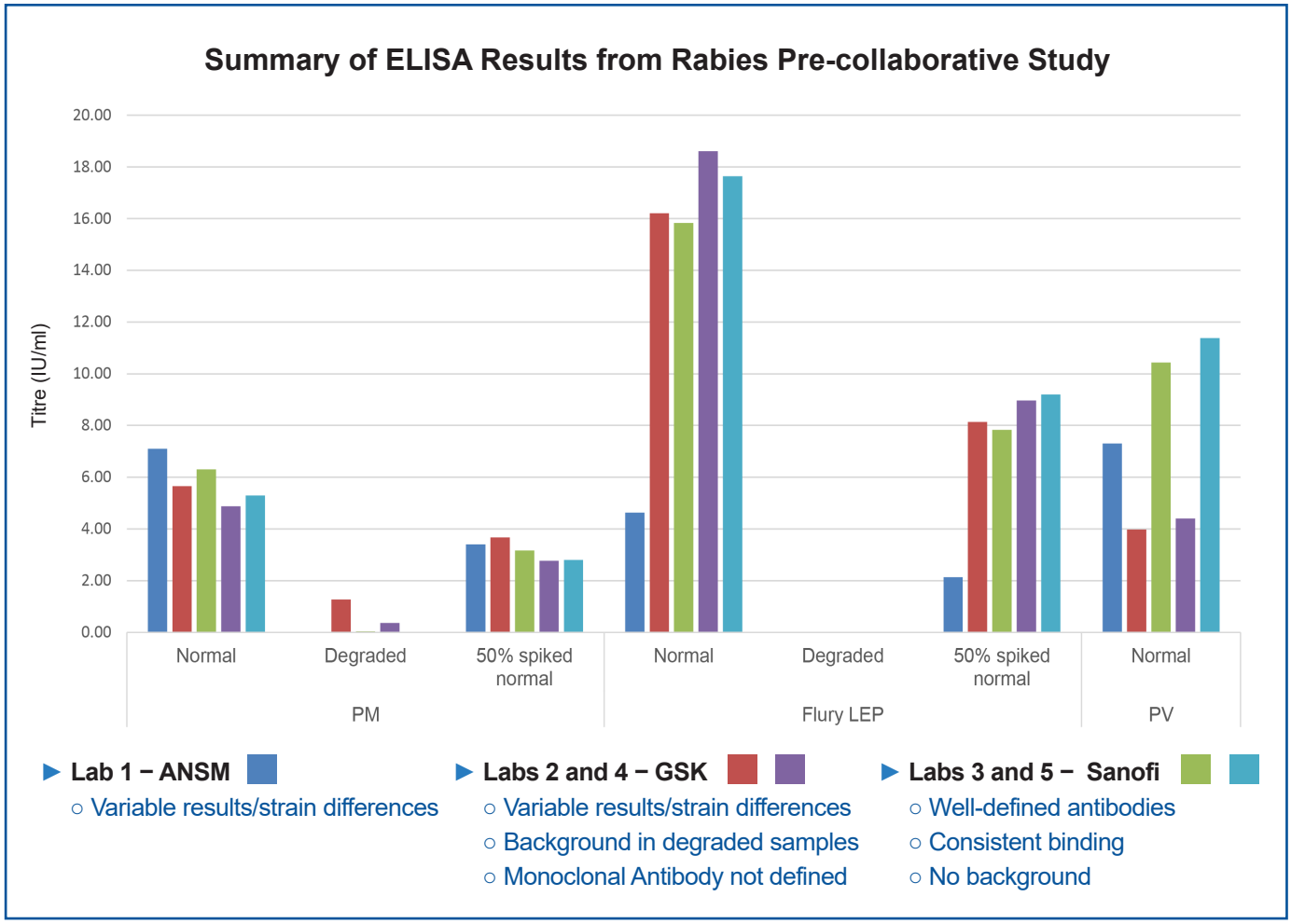
Here I just highlight this group of people came from all over the world ***[see slide #28]***. It was a really very interesting set of discussions. The outcome of that workshop was that everyone felt there were ELISA methodologies out there and that there were reagents starting to become available – and that everyone's homework was to go away and test out and come back again with what the best reagents were and what assays they could provide.

Three years later a second workshop was held, which was to define the alternate potency assay. The assay that was chosen: The working group determined that the Sanofi-Pasteur assay was appropriate for further development, because as I stated earlier, it clicked all of the things. The Sanofi-Pasteur ELISA monoclonal antibodies bind to conformational epitopes located on well-defined antigenic sites and the assay clearly discriminated potent from subpotent lots.

So then we all had homework to go back and do another set of studies looking at pre-validation work to look at the broad scope and ability for this ELISA assay. Most of that work was conducted by Sanofi.

<u>The Follow-up Studies</u>

Here I am just going to go very quickly through these. These are actually just the results from the pre-collaborative study that again, labs three and five were the FDA lab and the Sanofi lab, and that assay was chosen based on the antibodies, the consistent binding, and the lack of background.



This is just some information on the antibodies. I will highlight here that the first antibody is a site-two antibody, which is bright red in the protein at the bottom. So it is a conformational epitope, and the second antibody is one that is a strongly neutralizing linear epitope.

Prior to the global collaborative study – and I have already talked about this a little bit – Sanofi-Pasteur conducted a pre-validation study looking at as many strains as they could get globally ***[see slide #34]***. They were able to get strains from India, China, and South America, and so their results really showed broad applicability of the assay and that the study will now get turned over to the EDQM for final work.

My last slide just describes the aim of the study by the EDQM, and this is the preparatory phase. And even now – we have an assay that everyone agrees is a great assay – it is still not slated to be implementable until 2021.

I was a little disheartened to hear that because we have been working for so long, and why can't we just do it next year? But then you look at all of the efforts that have to go through.

So I think as we talk in this room and at the workshops this afternoon, we really have to think about the time involved in this as well. You can have a lot of great ideas, but implementation, especially on a global level, is really a whole other ball game.