From: Ivins, Bruce E Dr USAMRIID
Sent: Wednesday, March 08, 2000 12:50 PM
To: (b) (6)
Subject: RE: ELISA & TNA

Hi (b) (6)

Good to hear from you again. If we keep this correspondence going I must put you on my list of people to send "jokes" that I receive over email!

For (b) (6) email address, try either of the following:

(b) (6)

or

(b) (6)

I am accustomed to looking just at titers as well. However, it was thought by (b) (6) and others, that determining actual concentrations of anti-PA IgG would be more quantitative (therefore, I guess, more accurate and meaningful). There is a tendency by some around here to be rather anal-retentive about scientific experiments. We still do just titers for animals in routine experiments, but for stuff that's going to the FDA, exact concentrations of specific IgG will be determined.

Let me know if you'd like to be sent some of my "Yankee" humor.

- Bruce

-----Original Message-----
From: (b) (6)
Sent: Wednesday, March 08, 2000 12:05 PM
To: 'Bruce.Ivins(b) (6)
Subject: ELISA & TNA
Importance: High

Dear Bruce,

Good to have you at the other end of the email line.

One last question on that subject - do you know (b) (6) email address?

Change of subject - thanks for your help with that last one.

I send you the extract below in confidence. It is the response from the resident immunologist/serologist to what is needed in order to do the serology and TNA properly for the planned vaccine and primate trials. It through me into confusion! Firstly, I only became aware of this new view that one should be measuring ug/ml of anti-PA IgG rather than titer as I was reading on the plane coming over! But trying to get an explanation from (b) (6) (b) (6) - he was down here for some of the FDA planning meetings a month ago) of how this is done left me wondering in what way it was more precise than titers for measuring antibody response. And this standard serum thing below.

Is he going over the top a bit? I said we had used a running control for our tests, which was serum from a vaccinated individual. If we were running out of that, we ran it in parallel with another vaccinee serum which then became a control. I said we were more interested in changes with time than absolute values. He certainly makes me feel I have been very amateur in my approach to ELISA over these many years. How do you react to this?

> -----Original Message-----
> From: (b) (6)
>
> For the last several days I have been talking to the folks at USAMRIID
> and my staff trying to develop a laboratory plan for testing specimens
> for the human and animal studies.
>
> There is no standard reference serum for doing ELISA or TNA. This is
> amazing to me considering all the work and years of experience that
> has gone into evaluating this vaccine using serologic techniques.
> What would have to be done is have people get vaccinated (standard
> regimen, 0,2,4, 6 months, maybe 12 months) and then plasmaphorese
> them. After screening the plasma (we have always converted to serum
> at this step), pool, purify out the IgG fraction and then affinity
> purify anti-PA IgG. At this point the mass of the anti-PA would have
> to be determined to assign a ug/ml value for the reference. Next an
> independent measure of antibody done by ELISA would have to be run and
> the mass estimate and ELISA estimate would have to be is reasonable
> agreement. After the anti-PA values are assigned the serum needs to
> be lyophilized in small aliquotes and stored for use and distribution.
>
> The USAMRIID folks estimated that we plasmaphorese 20 people at about
> 500 ml per person and hopefully be able to get about 10 liters of
> starting material to purify.
>