LETTERS

Edited by **Jennifer Sills**

Clarifying samples in Zika analyses

IN RESPONSE TO the Zika outbreak and putatively related microcephaly cases in Brazil, many research groups in Brazil, North America, and Europe are studying the virus ("Evidence grows for Zika virus as pregnancy danger," G. Vogel, In Depth, 11 March, p. 1123). The simultaneous efforts to address this urgent matter have led to the use of some samples in multiple studies. There is no clear leadership or protocol to regulate access to patients, some of whom are also participants in studies of potential microcephaly causes unrelated to Zika, such as cytomegalovirus and genetic inbreeding (*1*). Some studies are reporting different results for the same set of samples. For example, Calvet *et al.* (*2*) and Oliveira Melo *et al.* (*3*) acknowledge overlap in the methods section. It is difficult to assess studies if we cannot determine whether, or to what degree, the cohorts are independent. Such confusion has already necessitated clarification of similar studies from different groups (*4*).

To address this problem, the World Health Organization could create a universal code for each baby with confirmed microcephaly and Zika infection, to be used by all research groups working with these data. The codes should include unique identifiers, generated by a competent government agency, that indicate the country and institution of diagnosis as well as a serial number for each patient. The World Health Organization could also provide a public database for Zika cases that would include the Zika codes as well as epidemiological information. These steps would allow individual cases to be identified in multiple studies while protecting privacy.

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Researchers studying Zika and microcephaly are using overlapping sets of data.

Animal-based antibodies: Obsolete

THE GLOBAL ANTIBODY industry produces an indispensable resource for biological, molecular, and cell scientists. Antibodies are harvested from immunized animals. The animals suffer side effects from the immunizations (*1*) and are, in some cases, mistreated (*2*). It is no longer necessary to compromise animal welfare: Since the mid-1990s, animals have not been required for antibody production (*3*). It is long past time to replace the use of animal-generated antibodies with nonimmunized recombinant antibodies.

Animal-Friendly Affinity reagents (AFAs) are antibodies that are generated by using recombinant technology in viruses or yeasts. The technology allows cloning of immunoglobulin gene segments, to produce antibody libraries with high diversity from which antibodies with desired specificities can be chosen. These are translated on the surfaces of cells or phage particles, and exposed to the target antigen, which selects a highly specific antibody, after which production can be scaled up within cell culture. AFAs are commercially available and can also be developed in individual laboratories. They have wide-ranging applicability as well as specificity and affinity—equal or greater to their animal-generated counterparts—to a huge repertoire of antigens. They also give researchers greater control over antibody properties, generation time, and cost (*4*). Thanks to AFAs, the use of animals has become obsolete.

EU Directive 2010/63/EU (*5*) requires the replacement of animals used in scientific procedures when alternatives exist. Yet, despite the maturation of a growing number of techniques to produce AFAs, antibody production using animals continues to be authorized. Twenty years ago, EU Member States were advised that "in the near future," antibody production "without prior immunization of [animals would] avoid the need to use living animals" (*6*). That prediction was correct. It is incomprehensible that such needless animal use continues.

There is little clarity about how many animals are used to produce antibodies. Only two EU Member State countries have published antibody production numbers. In 2013, the United Kingdom reported use of 9522 animals (*7*), and The Netherlands reported the use of 25,697 animals (*8*). The numbers do not include animals used to produce antibodies that were imported into those countries. Neither country has published the number of animals used for this purpose since 2013.

We recommend the following actions: Antibody production methods that use animal immunization should be replaced in EU Member States. Manufacturers outside the European Union should be required to adhere to European standards to qualify for import to Member States. An expert working group should be established to set up a roadmap for replacement. Programs should be implemented to ensure that animal-friendly antibody producers are fully supported. Subsequent reports from the Commission to the Council and the European Parliament on the statistics on the number of animals used for experimental and other scientific purposes should include data on the use of animals for antibody production as an independent category. These actions should be reinforced through international cooperation and national agencies that can execute government regulation and prevent outsourcing to regions where animal welfare is less well regulated.

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The line between science and politics

M. ENSERINK'S NEWS Feature "Peace of mind" (3 June, p. 1158) described Mohammad Herzallah's effort to establish a research oasis in the West Bank. Such a research center could benefit not only the Palestinians in the region, but the development of science throughout the Middle East. Enserink rightly highlighted the physical difficulties of getting such an oasis off the ground given barriers such as checkpoints and securities walls. However, he crossed the line into politics when he wrote, "Some hilltops are crowned with the modern contours of Israeli settlements, a major obstacle in the quest for peace."

Whether you agree with this statement or not, it does not belong in an article about establishing a new scientific endeavor.

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TECHNICAL COMMENT ABSTRACTS

Comment on "Multiple repressive mechanisms in the hippocampus during memory formation"

Rebecca S. Mathew, Hillary Mullan, Jan Krzysztof Blusztajn, Maria K. Lehtinen

Cho *et al.* (Reports, 2 October 2015, p. 82) report that gene repression after contextual fear conditioning regulates hippocampal memory formation. We observe low levels of expression for many of the top candidate genes in the hippocampus and robust expression in the choroid plexus, as well as repression at 4 hours after contextual fear conditioning, suggesting the inclusion of choroid plexus messenger RNAs in Cho *et al.* hippocampal samples.

Full text at http://dx.doi.org/10.1126/science.aaf1288

Response to Comment on "Multiple repressive mechanisms in the hippocampus during memory formation"

Jun Cho, Nam-Kyung Yu, V. Narry Kim, Bong-Kiun Kaang

Mathew *et al.* propose that many candidate genes identified in our study may reflect the events in the choroid plexus (ChP) potentially included in hippocampal samples. We reanalyze our data and find that the ChP inclusion is unlikely to affect our major conclusions regarding the basal suppression of translational machinery or the early translational repression (at 5 to 10 minutes). As Mathew *et al.* examined for a subset of genes at 4 hours, we agree that the late suppression may partly reflect the events in the ChP. Although the precise contribution of anatomical sources remains to be clarified, our behavioral analyses indicate that the late-phase suppression of these genes may contribute to memory formation.

Full text at http://dx.doi.org/10.1126/science.aaf2081

Animal-based antibodies: Obsolete

A. C. Gray, S. S. Sidhu, P. C. Chandrasekera, C. F. M. Hendriksen and C. A. K. Borrebaeck (July 28, 2016)

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