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Article

ICLAS LAQ Network for the Promotion of Animal Quality in Research

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Abstract

ICLAS Laboratory Animal Quality Network (LAQN) programs currently consist of the Performance Evaluation Program (PEP), which focuses on microbial monitoring by and for laboratory animal diagnostic laboratories, and the Genetic Reference Monitoring Program (GENRef), which provides assay-ready reference DNA for genetic testing of mouse strains. Since 2008, PEP has grown to become a truly international program with participating laboratories in 5 continents. Launched in 2016, GENRef currently distributes DNA from 12 common inbred mouse strains for use in genetic monitoring of locally inbred colonies as well as for genetic testing of stocks, particularly genetically engineered stocks, of uncertain origins. GENRef has the capacity to include additional strains as well as additional species. PEP and GENRef provide the reagents at cost, as a resource to the international scientific community, in the interest of improving research quality in an environment of growing concern for research quality, rigor, and reproducibility.

Key words: animal models, diagnostic testing, genetic testing, mice, rats, reproducibility of results, quality control, virus

BACKGROUND

Since its inception in 1956, a core objective of the International Council for Laboratory Animal Science (ICLAS) has been to promote international harmonization in the quality of research and of the animals involved in research. The goal of this harmonization is to mitigate confounding variables in research involving animal subjects both to enhance the quality and reproducibility of the science and to reduce the numbers of animals required to achieve rigor and validity. Animal health and well-being and the quality of research are influenced by many factors, including nutrition, environment, genetics, and disease status. Quality assurance refers to how a process is performed to assure quality. Quality control refers to the quality management procedures that fulfil quality requirements (eg, of accrediting organizations). Toward this end, ICLAS has contributed to and published guidance regarding laboratory animal nutrition[1] and laboratory animal nomenclature,[2,3] and LAQN current activities promote quality assurance in disease assessment and genetic monitoring of laboratory rodents.

In 1969, in conjunction with the World Health Organization, ICLAS established 2 areas where reference centers for laboratory

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animals were defined: Virus Reference Centers for Rodents and Histocompatibility Reference Centers for Mice, followed later by the addition of other laboratories for other species (ie, dogs and non-human primates) and biomarkers. In 1983, these reference laboratories were replaced by the ICLAS Monitoring and Reference Centers Program. Under this program, the Central Institute for Experimental Animals in Japan served as a monitoring center to promote breeding and maintenance of microbiologically and genetically standardized laboratory animals to standardize and harmonize research and testing procedures.

Several organizations have implemented the so-called "ring test," also called a ring trial or proficiency test, which is an inter-laboratory test to evaluate the performance of testing laboratories based on analyses of verified samples. These are established approaches to monitor the quality of analytical results, identify assays that need improvement, and serve as quality control measures to demonstrate competency to accreditation bodies. Examples of other regional quality control initiatives included the National Diagnostic Quality Assurance Program, based at the Rockefeller University (New York, NY, USA), supported by the National Institutes of Health in the United States from about 1978 to 1995, and the Quality Assurance Program of the Deutsches Krebsforschungszentrum, the inter-laboratory testing (ring-testing) program in Europe where the subscribing laboratories received the same unknown sample for testing. However, the objective of these programs was to reach a diagnostic consensus rather than to provide already confirmed samples to serve as reference specimens for laboratories.[4]

The ICLAS Monitoring and Reference Centers Program was active until 2006 when it was replaced by the current Laboratory Animal Quality Network (LAQN), which focuses on providing verified samples to research laboratories to independently assure the accuracy of their in-house diagnostic tests on rodent genetics and infectious agents.

LABORATORY ANIMAL QUALITY NETWORK

In 2004, after discussions with internationally recognized scientists in the field of health monitoring, a decision was made to replace the ICLAS Monitoring and Reference Centers Program with a new initiative more international in scope and able to serve as a truly transnational reference in the field of high-quality laboratory animal models.

These discussions led to the creation of the ICLAS Network for Promotion of Animal Quality in Research (LAQN) in 2006, whose founding members were Patri Vergara and Cecilia Carbone (ICLAS Governing Board members), Bill Shek (RADS, Charles River, USA), Lela Riley (RADIL, University of Missouri), Werner Nicklas (German Cancer Research Center), Esther Schoondermark (Radboud University, the Netherlands), and Marge Strobel (The Jackson Laboratory, USA).

The LAQN's mission was to develop programs to help achieve ICLAS's goal of improving the quality of research and the animals used in research in the areas of animal health and genetic monitoring. The programs developed under the umbrella of the LAQN were intended to have an educational component (organization of workshops and seminars) as well as to provide tools for the benefit of the laboratory animal community.

PERFORMANCE EVALUATION PROGRAM

LAQN's first initiative focused on microbial diagnostic testing for health monitoring and led to the creation of the ICLAS Performance Evaluation Program (PEP) in 2007.

PEP's aim was to improve health monitoring by providing well-characterized serum and microbiology samples to be used to assess the accuracy of testing by participating diagnostic laboratories. Network "member laboratories" prepare standardized sera and verified microbiological specimens and send them as unknown samples for analysis to any diagnostic laboratory ("participating laboratory") in the program. A comparison of the participant laboratory's results with the verified contents of the specimens, as detailed in a report (expected results) sent later by the LAQN, indicates the accuracy and/or sensitivity of the participating laboratory's assays.

Key features of PEP eligibility and participation (for participating laboratories) include the following:

1. The program is open to all diagnostic laboratories worldwide with no specific eligibility requirements;
2. Diagnostic performance is self-assessed, and participating laboratories are not asked to submit reports of their results to ICLAS or any other agency; and
3. The program is self-financed; participating laboratories pay in advance to cover the costs of sample production, shipment, and program administration.

IMPLEMENTATION OF PEP

Following a successful development stage,[5] PEP became fully operational in 2008 with 2 production member laboratories, Charles River USA (RADS) and RADIL (University of Missouri), and 9 participating laboratories. Since 2008, the network has incorporated 4 more production member laboratories (Central Institute for Experimental Animals, Japan; QM Diagnostics, Radboud University, Netherlands; German Cancer Research Center, Germany; Cerberus Sciences, Australia), and in 2010, the SIAL laboratory at the Universitat Autonoma de Barcelona (Spain) was established as the specimen storage and distribution center, thanks to a grant from the Spanish Ministry of Science.

PEP PARTICIPANTS

In its first 12 years, PEP has grown to become a truly international program with participating laboratories from 5 continents. Since 2008, more than 1000 specimens have been shipped worldwide, and PEP participants have grown from 10 in 2008 to an average of 24 for the last 5 years of the program. In 2020, the combination program was the most popular (15), compared with serology only (5) and microbiology only (3). A total 34% of participating laboratories were based in Asia, 33% in Europe, 21% in the United States, 8% in Oceania, and 4% in South America (Table 1).

PEP PROCEDURE

Current PEP Programs

Each laboratory participating in PEP can choose to join for serology and/or microbiology programs and receives 10 or 20 samples of positive sera and/or microbiology according to the program chosen (Table 2).

PEP Specimen Production

1. Network member laboratories produce standardized serology and/or microbiology specimens.
2. Specimen samples are sent to another network member laboratory for confirmation, that is, to verify that the target agent(s) can be detected. If the target agent is not detected

Table 1. PEP Participating Laboratories: 2008–2020 and the Type of Program Subscribed

● Serology ▢ Microbiology only ■ Combination ♦ Not participating

Participating Laboratories	08	09	10	11	12	13	14	15	16	17	18	19	20
001 EU	■	■	■	♦	♦	♦	■	■	♦	♦	♦	♦	♦
002 UK					■	■	■	■	■	■	♦	♦	♦
003 EU	■	■	■	■	■	■	■	■	■	■	■	■	■
004 EU	■	■	■	■	■	■	■	■	■	■	■	■	■
005 AU	●	■	■	■	■	■	■	■	■	■	■	■	■
006 US	●	●	●	●	♦	♦	♦	♦	♦	♦	♦	♦	♦
007 US	■	■	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	■
008 AS	■	■	■	■	■	■	■	■	■	■	■	■	■
009 AU	■	■	■	■	■	■	■	■	■	■	■	■	■
010 EU							●	●	●	●	♦	●	▢
011 EU	●	■	■	■	■	■	▢	▢	▢	▢	▢	▢	▢
012 EU	●	●	●	●	●	●	●	●	●	●	●	●	●
013 US		■	■	♦	♦	■	■	■	▢	▢	♦	♦	♦
014 AS			●	■	■	■	■	■	♦	■	■	■	■
015 AS			●	■	■	■	■	■	■	■	■	■	■
016 US			■	■	■	■	■	■	■	■	■	■	■
017 US			■	■	■	■	■	■	■	■	■	■	■
019 AS				■	♦	■	♦	♦	■	♦	♦	♦	♦
018 SA					■	■	■	■	■	■	■	■	■
020 EU					■	■	■	■	■	■	■	■	■
021 EU					■	●	●	●	■	●	●	●	●
022 AS					■	●	♦	■	♦	♦	♦	♦	♦
023 US					●	●	●	●	■	■	●	●	●
024 EU					▢	▢	▢	▢	▢	♦	♦	♦	♦
025 AS						▢	■	■	■	■	▢	▢	●
026 EU					■	♦	♦	■	♦	♦	♦	♦	♦
027 AS						■	■	■	■	▢	▢	▢	▢
028 AS						■	♦	♦	■	♦	♦	♦	♦
029 AS						■	♦	♦	♦	■	♦	♦	♦
030 EU							■	■	■	■	■	■	■
031 EU								▢	♦	♦	♦	♦	♦
032 AS									●	■	●	●	●
033 EU									■	♦	♦	♦	♦
034 EU									▢	▢	♦	♦	♦
035 EU									▢	♦	♦	♦	♦
036 AS									■	■	■	■	■
037 AS									●	♦	♦	♦	♦
038 AS										●	♦	♦	♦
039 EU											■	■	■
040 US											■	■	♦
041 AS												■	♦
Total Serology only	4	2	4	2	2	4	4	4	4	4	**4**	**5**	**5**
Total Microbiology only	0	0	0	0	1	2	2	3	5	4	3	3	3
Total Combination	6	9	1	1	16	18	1	1	20	18	15	16	15
Total Participants	**10**	**11**	**14**	**13**	**19**	**24**	**23**	**25**	**29**	**26**	**22**	**24**	**23**

or if a specimen proves positive for an unintended agent, the batch would be rejected.

3. When specimens are confirmed, network member laboratories send specimens to the distribution center for distribution to participating laboratories.

Characterization and Quality Control of PEP Specimens

Table 3 summarizes the steps to produce and validate PEP specimens before incorporating them into the program for distribution to the participating laboratories.

Table 2. Optional Programs Inside PEP

Programs	Shipments/Specimens
Serology only	1 annual shipment of 10 sera specimens
Microbiology only	1 annual shipment of 10 microbiology specimens
Combination	1 annual shipment of 10 sera and 10 microbiology specimens

PEP, Performance Evaluation Program.

Table 3. Production, Characterization, and QC of PEP Specimens

Production and Characterization of PEP Specimens	PEP Specimen QC
• All specimens generated under strict conditions and rigorously characterized. • Infectious agents are obtained from known sources and sequenced to confirm identity. • Experimental animals are inoculated, and the serum and relevant tissues are collected and aliquoted for use as standardized specimens. • Aliquoted specimens are evaluated by 2 laboratories to confirm quality	• Acceptance criteria: Pure and Potent • Immune serum – Seropositive to inoculated pathogen only – Moderate to strong reaction by standard assays • Infectious specimen – Free of extraneous pathogens – Easily detectable concentration of pathogen

PEP, Performance Evaluation Program; QC, quality control.

Table 4. Steps of PEP Application and Participation by Participating Laboratories

June–December, year 1	January–May, year 2
1. Participants submit PEP application form	5. Participants receive specimens from PEP distribution center by international courier
2. Participants receive invoice	6. Participants analyze specimens
3. Participants pay participation fee by bank transfer	7. Participants request and are sent expected results (Fig. 2)
4. Participants receive PEP Participation Certificate	8. Participants compare their results with expected results to determine accuracy and/or sensitivity of their assay performance
	9. Participants are requested to provide feedback regarding any concerns or discrepancies in their results. These responses are voluntary and are presented anonymously in PEP reports.

PEP, Performance Evaluation Program.

PEP SELF-ASSESSMENT

PEP was designed as a self-assessment program for the diagnostic laboratories. Participating laboratories can use the accuracy of their results to check their own systems and to fulfil the requirements of certifying agencies such as ISO. Table 4 summarizes the steps of PEP application and participation by participating laboratories. After completing their analyses, participating laboratories request the expected results for their samples. An example is shown in Figure 1. Although participating laboratories are not obliged to send back a report to the LAQN, any feedback is appreciated because it helps to detect any problems and gives the opportunity to exchange information with the producer laboratories.

FUTURE CHALLENGES FOR PEP

Quality assurance and quality control are essential in diagnostic laboratories. PEP provides the means for member laboratories to self-assess the quality of their assays. Our current aim is to increase the number of participating laboratories and to facilitate improvement in laboratory animal diagnostics.

PEP and diagnostic laboratories are facing 2 major challenges. First, the pathogens affecting laboratory animals are changing, as exemplified by the current rarity of some pathogens that were common 20 years ago, for example, Sendai virus, and the emergence of potentially new pathogens by the increased use of immunodeficient mice and xenotransplants. For this reason, the PEP library is open, and, although it is difficult to have a complete inventory of potential new findings, PEP also tries to include less common agents isolated by the producer laboratories.

Second, PEP standard samples are increasingly expensive to produce, particularly serum samples because they imply the infection of new animals. PEP producer laboratories are investing their own resources and their own quality assurance and quality control and are key participants in the quality of biomedical research.

GENETIC REFERENCE MONITORING PROGRAM

In parallel to the development of PEP, in 2008, the network began to develop a genetic monitoring program. The principal aim was to address the issues of genetic quality assurance arising from the dramatic increase in rodent strains and stocks and the use of genetically modified animals. Genetic quality assurance was recognized as crucial for ensuring consistency and reproducibility in experimental results both within and across research institutions. Of note, researchers in many regions cannot readily purchase stocks or strains of laboratory mice or rats of known genetic background from quality commercial vendors. The logistics and costs of international transport are often too daunting, given the challenges of long distances, connecting flights, weather and temperature extremes, insufficient oxygen in cargo holds, flight and customs delays, and the attendant

ICLAS Diagnostic Laboratory Performance Evaluation Program
Expected Results for Distributed Specimens

Distribution # 50: PEP2019 Serology and Microbiology

50-1	Mouse serum diluted 5-fold in PBS	0.50 mL x1	Microbial Antibodies	Adenovirus FL Antibodies
50-2	Mouse serum diluted 5-fold in PBS	0.40 mL x1	Microbial Antibodies	CAR Bacillus Antibodies
50-3	Rat serum diluted 5-fold in PBS	0.50 mL x1	Microbial Antibodies	PVM Antibodies
50-4	Rat serum diluted 5-fold in PBS	0.50 mL x1	Microbial Antibodies	Pneumocystis carinii (P. Jirovecii) + CARBAntibodies
50-5	Rat serum diluted 5-fold in PBS	0.50 mL x1	Microbial Antibodies	Sendai virus Antibodies
50-6	Rat serum diluted 5-fold in PBS	0.40 mL x1	Microbial Antibodies	Rat parvovirus (KRV) Antibodies
50-7	Mouse serum diluted 5-fold in PBS	0.50 mL x1	Microbial Antibodies	Polyoma virus Antibodies
50-8	Mouse serum diluted 5-fold in PBS	0.50 mL x1	Microbial Antibodies	EMCV Antibodies
50-9	Rat serum diluted 5-fold in PBS	0.50 mL x1	Microbial Antibodies	Reovirus Antibodies
50-10	Mouse serum diluted 5-fold in PBS	0.50 mL x1	Microbial Antibodies	TMEV Antibodies
50-11	Rat spleen homogenate	0.50 mL x1	Virus	Toolan's H1
50-12	Bacterial culture	0.50 mL x1	Bacteria	Streptococcus agalactiae
50-13	Bacterial culture	0.50 mL x1	Bacteria	Serratia marcescens
50-14	Bacterial culture	0.50 mL x1	Bacteria	Rodentibacter heylii
	Mouse feces	0.50 mL x1	Bacteria	Helicobacter rodentium
50-16	Lung homogenate	0.50 mL x1	Virus	PVM
50-17	Bacterial culture	0.50 mL x1	Bacteria	Escherichia coli
50-18	Rat fluid diluted 10-fold	1.0 mL x1	Virus	SDAV
50-19	Bacterial culture	0.50 mL x1	Bacteria	Aeromonas hydrophila

Figure 1: Example of expected results for the 2019 PEP Combination Program.

risks of physical trauma or pathogen contamination en route, if the animals survive the journey. Thus, many facilities breed their own animals but lack the means to assess and correct for genetic drift and mutation or to refresh the lines with strains of confirmed genetic background.

GENRef program eligibility and participation has 3 key features in common with PEP:

1. Open to any research laboratory worldwide with no specific eligibility requirements;
2. Monitoring is self-assessed; and
3. Each participant pays in advance to cover the costs of specimen production, shipping, and administration.

GENRef was developed in 2 phases: first, a focus on education and training to increase awareness of the importance of genetic quality monitoring; and second, the establishment of a self-assessment genetic monitoring program.

IMPLEMENTATION OF THE GENREF PROGRAM

Education and training on the importance of genetic quality monitoring started in 2010, with presentations at various laboratory animal science conferences such as American Association for Laboratory Animal Science, Asian Federation of Laboratory Animal Science Associations, and Federation of

European Laboratory Animal Science Associations in conjunction with 2 papers published in 2013: 1 in *Mammalian Genome*[6] and the other as a congress paper at the 12th FELASA-SECAL Congress.[7]

In 2016, the GENRef program was launched and has since provided 118 DNA specimens to 13 GENRef participants, mainly in the Americas and Asia, in accordance with the following aims and procedures.

Aims and Procedures

Reference DNA from commonly used rodent strains and stocks is made available to enable research institutions worldwide (program participants) to

1. assess if their colonies are genetically sound and representative of the expected genetic background; and
2. assess animals of mixed or uncertain backgrounds, for example, genetically engineered animals developed from multiple strains.

Species

The initial focus is on the genetic monitoring of rodents (mice and rats), both inbred and outbred (or closed colony animals), although to date, the program has only provided inbred mouse DNA.

DNA Strains

The program currently provides reference DNA from 12 common inbred strains/sub-strains of laboratory mice:

- J strains: C57BL/6 J (reg. #664; BALB/cJ (reg. #651); NOD/LtJ (reg. 1976); A/J (reg.#646)
- Tac strains: C57BL/6NTac; BALB/cAnNTac; C3H/HeNTac; 129S6/SvEvTac
- Jcl strains: C3H/HeJJcl; DBA/2NJcl; FVB/NJcl

GENREF SPECIMEN PRODUCTION

1. DNA providers: DNA for the first batch of samples was provided by 3 internationally recognized breeders (donor breeders): The Jackson Laboratory, Taconic Biosciences, and Central Institute for Experimental Animals. All costs for this first batch of 100 units per strain were covered by the breeders as a donation to the program.
2. DNA production: Donor breeders isolated DNA from 4 mice of each strain from the following tissues: tail, lungs, heart, and kidneys. DNA was extracted and placed in tubes (units) at concentrations of 25 ng/μL and a total of 10 μL or 250 ng/10 μL.
3. DNA confirmation: Donor breeders provide DNA samples to another donor breeder or to an ICLAS Network member laboratory for confirmation.

FUTURE CHALLENGES FOR GENREF

The GENRef program seeks to raise recognition of the need to assess and monitor genetic backgrounds of research mice and to increase awareness and participation in the GENRef program. Examples of sub-strain variations and research complications related to complex, under-characterized, or otherwise uncertain genetic backgrounds are increasing.[8,9] ICLAS will continue to provide information on its website on the equipment, reagents, and protocols needed to use the GENRef program as well as via regional, national, and international meetings and via educational programs and publications.

The number of DNA strains offered will be expanded to ensure that most used strains around the world are available. For example, due to the increase of CRISPR techniques, there has been an increase in C57BL/6 background mice and less use of other strains. Although GENRef already includes 2 C57BL/6 sub-strains, additional C57BL/6 sub-strains will strengthen the program.

CONCLUSION

Both PEP and GENRef are established programs with functional capacity to expand in response to increasing concerns for research quality, rigor, and reproducibility and in response to increasing demands for Quality Assurance resources from diagnostic laboratories and research programs.

Further details about the ICLAS LAQ Network programs and publications as well as application forms to participate in the PEP and GENRef programs can be found at the ICLAS website at www.iclas.org.

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