

# **Validation of PCR-Based Assays for Detection of Invasive Aquatic Species and Laboratory Accreditation: Report of the ISAC EDRR Subcommittee, eDNA Working Group**

---

## **ABSTRACT**

The charge of the National Invasive Species Council (NISC) is to ensure that Federal programs and activities to prevent and control invasive species are coordinated, effective and efficient. The Invasive Species Advisory Committee (ISAC) functions to provide advice and recommendations to NISC on invasive species matters. ISAC's Early Detection and Rapid Response (EDRR) subcommittee is petitioning the National Academies of Science (NAS) to review activities across Federal Agencies involving development and use of Polymerase Chain Reaction (PCR) and other molecular assays for use in regulatory/legal decisions relative to aquatic invasive species (AIS). Such review will allow NAS to develop recommendations for validation of PCR and other molecular technology assays before field use to detect aquatic invasive species. The EDRR wishes to provide reasonable and sound advice to federal agencies specifically and others regarding the methods and paths for bridging from research bench to regulatory decision. We note previous NAS efforts to advise the FBI on the PCR assay developed during the investigation of the anthrax letters and most recently testimony to US Congress regarding Homeland Security's BioWatch program for aerosolized bioterrorism agents in urban areas.

Natural resource agencies may struggle to make the most appropriate management decisions regarding AIS due to often inadequate traditional methods for detection of early invasions. Thus, increased interest in genomic-based tools for identification, detection, and monitoring of AIS species has developed mostly due to their high level of detection sensitivity. This growing interest has prompted widespread speculation on future availability of inexpensive and rapid molecular assays. Such assays are envisioned to enable earlier detection; rapid identification; and timelier monitoring of aquatic invasive pests. Recent application of genomic assays such as PCR for early detection of AIS not standardized across laboratories has led to inconclusive, inaccurate, incomplete, and conflicting results. Thus, application of the concepts of assay validation and laboratory accreditation are urgently needed to standardize genomic testing available to regulatory decision makers and private industry regarding AIS. To date, uncertainty of genomic assay results have, in many situations, led agencies responsible for managing AIS to require cumbersome, delaying, separate, and independent verification of the initial assay results before taking action. Regulatory officials and industry representatives have expressed the need to ascertain the validity of an assay's performance in terms of sensitivity, specificity, accuracy, and robustness, as well as establishing an accreditation program to benchmark laboratory performance.

---

<sup>1</sup> Prepared by the ISAC EDRR subcommittee working group: D. Starling, S. Phillips, N. Stone, and R. McMahon.

## **BACKGROUND**

For this discussion, an “assay” is any test or method utilized to convert or amplify some environmental activity or event that permits detection. However, assays must be reliable when used in a specific situation. The one area of recent development and rapidly expanding assay activity that has not been universally addressed with these concepts and approaches is use of Polymerase Chain Reaction (PCR) detection of AIS. The aquatic environment is relatively difficult to explore and define. The activities of AIS entering, intruding, or invading aquatic habitats often goes unnoticed until a population has been established (National Invasive Species Council 2003). First detection of an aquatic invasive species has historically often occurred far beyond the point-of-no-return for extirpating the infestation.

On June 14, 2011, a discussion of genomic techniques for AIS detection was held during a meeting of the EDRR subcommittee of ISAC. This discussion centered on validation requirements that might apply to molecular genomic technology, specifically polymerase chain reaction, used to detect AIS including the context of various intended uses. A unique feature of this discussion was a focus on developing case studies that would serve as “what if” or real world hypothetical scenarios on which various stakeholders could comment and provide insight for those developing new molecular genomic assays. The discussion was designed to augment the activities of the EDRR subcommittee charge to advise ISAC on issues and recommendation important to the NISC mission.

Many federal agencies and industries recognize the potential of PCR for AIS early detection and control. For this reason, the EDRR subcommittee established a Working Group to identify needs and concerns related to current practices using PCR. These include: 1) the reliability of results developed from genomic assays are not subjected to a defined and rigorous framework of validation; 2) practicing laboratories are acting without levels of quality control and quality assurance capabilities at a suitable level of performance for accreditation; and 3) regulatory decisions made without reliable information because of deficiencies in the assays utilized (Frischer et al. 2011). At its June 2011 meeting, the EDRR presented a draft Whitepaper outline as a first step in defining a framework in the process that would result in regulatory decision makers having reliable results from PCR assays. It is foreseen that this Whitepaper will lay the foundation for formal systematic and orderly assessment of PCR assays before results are used in making decisions by regulatory agencies. It is hoped that this effort will make the validation method more defined, transparent, and understandable in order to hasten the development, optimization, and validation of PCR assays that have a great potential to enhance our nation’s efforts in preventing, controlling, and monitoring AIS.

A crucial step in the development of field useable, molecular genomic assay(s) like PCR is determining analytical performance parameters that support regulatory decisions so that the results are reliable source(s) of information for decision-makers. Genomic molecular-based assays developed and validated on novel or complex platforms (such as micro-array or other multiplex assays) have not been proficiently established in the context of field use. Therefore, there has been some concern, uncertainty, and indeed, disagreement among researchers and developers about the type and depth of data needed to validate tests for field uses (Darling and Mahon 2011).

At the June 2011 ISAC meeting, presentations were given as background for ISAC members from scientists developing PCR (Dr. John Darling, EPA; Dr. Kevin Kelly, BOR) and a regulator involved in evaluating and licensing PCR Test Kits for the US animal disease test-kit market (Dr. Larry Ludemann, APHIS VS, CVB).

### **Overview of the Reasoning for Requesting Validation Requirements for PCR Assays Used in the Detection, Control, and Monitoring of AIS.**

There are numerous concerns regarding the reliability of results from assays conducted without appropriate validation of the method or definition of minimum laboratory requirements in key areas regarding performance. These concerns are especially poignant for assays used to determine the presence of AIS that may not be detectable by traditional investigative methods. PCR assays may provide the relative sensitivity to invoke a paradigm change in the way AIS are detected, controlled, and monitored. However, consequences of misdirected trust in an inappropriately developed and unvalidated assay could be far more damaging, destructive, and long lasting than any amount of damage caused by the arrival and establishment of an invasive species. This request for review of possible validation requirements for PCR and other genomic-based assays is not made to provide a simplified “preflight” checklist for evaluating molecular assays to be used in “detecting the presence” of AIS. Rather, this request’s intended outcome is to offer a useful framework of

definitions, considerations, and description of steps consistent with development, optimization, and validation of assays that provide truly useful and reliable information. Results from validated environmental DNA assays could then be regarded as useful information in making decisions regarding AIS prevention, control, and monitoring activities. However, without suitable validation of assay methodology and accreditation of the laboratory, the results may be misleading, and incorrect as well as damaging and fraudulent.

Currently, at least two federal agencies have some level of regulatory control regarding PCR assays that are developed and validated for marketing in the United States. The Federal Drug Administration (FDA) is responsible for enforcement of the Federal Food, Drug, and Cosmetic Act that covers *in vitro* diagnostic devices (IVD's) which are a subset of medical devices "intended for use in the diagnosis of disease and other conditions, including determination of the state of health, to cure, mitigate, treat, or prevent disease or its sequela"<sup>2</sup>. The Animal and Plant Health Inspection Service through the Center for Veterinary Biologics regulates the licensing and sale of diagnostic kits used in detecting animal diseases under the authority of the Virus Serum Toxin Act. Both agencies are involved in assuring that commercially available kits for running assays are safe, effective, reliable, and truthful in their label claims.

There is currently no required independent or regulatory oversight of laboratories conducting and performing PCR assays for AIS through the use of in-house reagents, protocols, and technologies. There are numerous "quality" concerned organizations which orientate their policies and philosophies towards the International Committee on Harmonization. These "quality" associations/organizations are voluntary. Membership brings recognition of a laboratory's effort to conform to quality standards in several areas important to reliable and reproducible laboratory operations. Generally inspections are conducted of member laboratories by a team of experts. Each team member can be specialized in some area of concern to the quality standards being verified. Areas of operation commonly observed, reviewed, and records audited can include facilities, equipment, personnel, protocols, and references, mechanisms of internal control and direction, etc. There are areas of exception in some regulatory programs for the prevention, control, and eradication of animal disease where participation may need to meet mandatory standards for facilities, equipment, and personnel. Many protocols in these regulated laboratories are standardized in accordance with international trade agreements or other legally binding documents. Personnel are encouraged to read the various "Uniform Methods and Rules" used when testing for pathogens of commercial and economic significance. It appears that human and animal health is well on its way to utilizing reliable assays for information regarding disease. There is also a system in place for plant health certification by way of testing for plant pathogens. In contrast, there does not appear to be any systematic or regulatory framework and oversight for environmental health, disease, or pest issues such as AIS.

Due to their potential negative economic and ecological impacts, one may question why decision makers would attempt to make decisions regarding AIS based on results from unvalidated assays. Recently, the US Supreme Court heard testimony about the use of a PCR assay for detecting invasive Asian Carp as "the best we have". Could the "best we have" be devastatingly wrong if there is a deficiency of solid science or a lack in validation of the assay or accreditation of the laboratory before it is applied in a real life situation? The simple and unequivocal answer is "yes"!

---

<sup>2</sup> Safety for IVDs is conceptualized as the benefit of a test result outweighing its risk, when the test is used as labeled. Safety can be connected to analytical performance, because the device developer must ensure that adequate instructions for use are provided, that the correct population for testing is identified, and that the expected measurement performance parameters are established. Effectiveness refers to evidence that the test result is clinically important when the test performs according to its labeling. This can be connected to the ability of the test to contribute to meaningful clinical decision-making. The type of supporting evidence required to establish a reasonable assurance of safety and effectiveness varies by device, but in general the test developer must show adequate, often statistically assessed, analytical and clinical performance of the device when used according to instructions and for its claimed intended use.

## **Request for Help by Expert Evaluation of the PCR Assay Validation and what Laboratory Standards to Expect in Generating Reliable Laboratory Reports of Results about Invasive Species.**

The National Academies of Sciences appears well positioned to evaluate this area of science and how it is being applied in decision making that determines action taken regarding AIS. There are at least two described occasions where NAS or its sections have been helpful in evaluating federal agency activities surrounding the development without validation of a molecular assay that was implemented out into field use. Both cases, (the first on the anthrax letters of 2001 involving the FBI's labs (National Research Council 2011); and, the second in Homeland Security) there is testimony by NAS experts to Congressional hearings<sup>3</sup> about installation of air sampling stations for early detection of bioterrorism agents in urban areas (National Research Council 2011). In both situations, NAS attention was requested in part to evaluate the effectiveness of assays without validation. Therefore, the EDRR subcommittee respectfully requests that ISAC recommends to NISC that they support and encourage the National Academy of Sciences to undertake a review of the reliability and effectiveness of PCR and other molecular genomic applications for detecting AIS with an eye towards establishing appropriate validation and reliability standards for this new and potentially invaluable tool in the prevention and control of AIS.

### **LITERATURE CITED**

- Darling, J.A., Mahon, A.R. 2011. From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environ. Res.* doi:10.106. Available at: [http://www.doi.gov/NISC/global/ISAC/ISAC\\_Minutes/2011/PDF/Darling\\_Mahon\\_Article.pdf](http://www.doi.gov/NISC/global/ISAC/ISAC_Minutes/2011/PDF/Darling_Mahon_Article.pdf). Accessed 10-31-2011.
- Frischer, M.E., Nierzwicki, S.A., Kelly, K.L. 2011. Reliability of early detection of *Dreissena* spp. larvae by cross polarized light microscopy, image flow cytometry and polymerase chain reaction assays. Results of a community double-blind round robin study (Round Robin Study Phase II). U.S. Department of the Interior, Bureau of Reclamation, Technical Service center, Denver, CO. 29 pp. Available at: <http://www.musselmonitoring.com/Reports/RRII%20Final%20Report%20%282010%29.pdf>. Accessed 10-31-2011.
- National Invasive Species Council. 2003. General Guidelines for the Establishment and Evaluation of Invasive Species Early Detection and Rapid Response Systems. Version 1. U.S. Department of the Interior, Washington, D.C., 16 pp. Available at: [http://www.invasivespecies.gov/global/EDRR/EDRR\\_documents/Guidelines%20for%20Early%20Detection%20&%20Rapid%20Response.pdf](http://www.invasivespecies.gov/global/EDRR/EDRR_documents/Guidelines%20for%20Early%20Detection%20&%20Rapid%20Response.pdf). Accessed 10-31-2011.
- National Research Council. 2011. Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Letters. The National Academies Press, Washington, D.C. 210 pp. Available at [http://www.nap.edu/catalog.php?record\\_id=13098](http://www.nap.edu/catalog.php?record_id=13098). Accessed 10-31-2011.

---

<sup>3</sup> Testimony of Tara O'Toole before the House Subcommittee on Homeland Security Appropriations, on Biosurveillance, April 16, 2010. [http://www.dhs.gov/ynews/testimony/testimony\\_1271436311919.shtm](http://www.dhs.gov/ynews/testimony/testimony_1271436311919.shtm) which refers to the NAS report, *BioWatch and Public Health Surveillance: Evaluating Systems for the Early Detection of Biological Threats*. The NAS report can be found online at [http://www.nap.edu/catalog.php?record\\_id=12688](http://www.nap.edu/catalog.php?record_id=12688) – accessed 20110914