Request for Applications

Detection of Gene Doping

1. Background

The World Anti-Doping Agency (WADA) was established in 1999 as an international independent agency to lead a collaborative worldwide movement for doping-free sport. WADA's governance and funding is based on equal partnership between the sport movement and governments of the world. WADA's primary role is to develop, harmonize and coordinate anti-doping rules and policies across all sports and countries. Our key activities include scientific and social science research; education; intelligence and investigations; development of antidoping capacity; and monitoring of compliance with the World Anti-Doping Code.

Science is key to driving advances in anti-doping. Innovative research leads to the identification of new doping trends, new substances, new doping methods and new detection approaches. WADA funds scientific research projects to develop and optimize analytical tools for the detection of use of prohibited substances and doping methods within sport populations.

WADA is pleased to launch a request for applications (RFA) that builds on previous funding for development of gene doping detection methods, which led to implementation in WADA-accredited laboratories of a sensitive analytical method using PCR. The goal of this RFA is to support projects that optimize and validate analytical tools to detect nucleic acids used for gene doping, incorporating technology platforms that can scale to detect a range of sequences in many samples without a loss of sensitivity relative to the current PCR approach.

2. Research topics and eligibility of research proposals

Applications eligible for this RFA for the direct detection of gene doping include but are not limited to:

a) **Sequencing-based methods**, which may include
   - Target-enrichment (e.g., probe hybridization) for specific sequences;
   - Cell-free DNA sequencing (i.e., targeted or untargeted);
   - Other DNA sequencing approaches adapted to detect gene doping, such as long-read sequencing, whole exome or whole genome sequencing; or
   - RNA sequencing adapted to detect specific foreign sequences found in mRNA therapeutics.

b) **Multiplexed CRISPR-based methods** for targeted nucleic acid detection.

At a minimum, eligible research plans should include the following:

- Optimization and validation of sequencing or CRISPR-based approaches to detect gene doping, including sensitivity to a range of nucleic acid sequences;
- The ability to detect different transgenes or related sequences used in gene doping methods and to be readily extended to detect new sequences (e.g., viral or non-viral vectors, regulatory sequences, etc.);
- Evaluation of sensitivity and specificity; and
- Evaluation of appropriate controls and blinded samples, including samples from clinical or pre-clinical studies that involved gene transfer.

Full applications invited following an expression of interest should also include the following:

- A summary of the method as it currently stands (e.g., sensitivity, read length and sequencing depth of coverage for sequencing methods, etc.) and anticipated improvements;
- Comparison with published PCR-based tests to detect *EPO* gene doping;

- Consideration of strengths and limitations relative to alternative approaches, such as a discussion or experimental evaluation of strengths and limitations of any target enrichment steps relative to alternative target enrichment approaches (e.g., hybrid-capture vs PCR);
- An explanation of how the test is expected to be used for doping control (e.g., in combination with screening procedures, such as immunological approaches to detect gene doping, or other confirmation procedures);
- A plan to transfer the method to WADA-accredited laboratories for implementation in routine anti-doping testing;
- An estimated cost per sample to provide the test for doping control, considering direct costs and uncertain or variable test volume;
- Timelines for each activity included in the proposal and estimated timelines for any other method development activities anticipated to extend beyond the term of the project; and
- Inter-laboratory reproducibility, if appropriate for the analytical validation plan.

Applicants are also encouraged to consider the following, if relevant:

- Detection of transfer of small genes with intact introns (e.g., intact *EPO* or *GH1*);
- Detection of nucleic acids with chemical modifications or with sequences that have been modified to avoid detection by current gene doping detection tests;
- Post-administration detection times;
- Multiple sample matrices (e.g., whole blood, plasma, serum, dried blood spots);
- Appropriate samples for WADA's External Quality Assessment Scheme;
- Cost relative to alternative approaches; and
- Procedures to address any secondary findings of pathogenic variants in medically actionable genes.

3. Applicants

Collaboration between research groups is encouraged, including integration of expertise in both anti-doping science and molecular diagnostics. All projects should demonstrate engagement of anti-doping laboratories (i.e., WADA-accredited laboratories), which bring expertise in methods for sample collection, test development, test validation, and ongoing test performance in compliance with the International Standard for Laboratories; engagement with anti-doping laboratory scientists during development of the research plan will help ensure practical applicability of the technology platform.

If inter-laboratory reproducibility is included in the proposal, at least two independent laboratories should participate in the study. These may include WADA-accredited anti-doping laboratories with access to appropriate equipment or other ISO/IEC 17025-accredited laboratories that may be eligible for WADA approval to perform specific methods under a subcontracting arrangement with a WADA-accredited laboratory.

4. Parameters

WADA will invest a maximum of USD 500,000 in an individual project if costs are well justified.

5. Application and decision process

Researchers interested in this RFA are invited to submit expressions of interest (EOI) **by 31 March 2023** (23:55 GMT) using the WADA Grants platform.

EOIs will be reviewed in April 2023. Those that are aligned with the specified research priorities and submitted by teams with relevant expertise will be invited to submit full applications on the WADA Grants platform, including complete project descriptions. Where applicable, additional guidance from subject experts may be provided to

inform applications from eligible applicant teams. Applicants can expect to receive a reply to their EOI by the end of May 2023.

EOIs and full applications must be submitted in English. All other relevant documents should be translated if the originals are in a language other than English. When an applicant is invited to submit a full application, the following enclosures will be requested by 30 June 2023:

a) A project description (maximum five pages) including objectives, methodology, experimental design, timelines, preliminary results and relevant bibliographical references;
b) Information about the researchers (curriculum vitae), their home institution and resources;
c) A detailed budget;
d) *For research involving human subjects and/or human samples (including existing material), a copy of the local ethics committee approval, participant information letter and consent form.
e) *For research involving animals: a copy of animal care committee approval.
*If these documents are pending at the time of submission, WADA may approve the proposal for funding contingent upon ethics review; however, these documents will be required once the grant is approved.

Applicants are encouraged to contact WADA (science@wada-ama.org) for assistance with composition of the research team and access to biological samples, as well as other technical aspects.

Full applications will be reviewed via a process that will involve independent experts and members of WADA's Gene and Cell Doping Expert Advisory Group (GCDEAG). Applicants will receive decisions about full applications by October 2023.