

eDNA proficiency testing

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ADAS has taken part in the new eDNA proficiency testing scheme facilitated by Natural England and has CORRECTLY IDENTIFIED ALL 'blind' samples as positive, negative, or inhibited.



From the 2017 survey season onwards Natural England will **only** accept eDNA results from laboratories participating in the proficiency testing scheme to support GCN license applications and post-development presence or absence monitoring for mitigation licences. Our clients and ourselves have (rightly) been calling for this scheme since eDNA analysis was first approved by Natural England in 2014 when we first offered this service.

The aim of this test is to provide evidence of the robustness of the technique and ensure that laboratories offering this analysis provide a good quality service. This test will also allow Natural England to understand the performance of laboratories providing an analytical service in the UK. Any laboratories who do not correctly assign 'blind' samples will be given the opportunity to improve their standards and re-take the test. Seven 'blind' samples were sent out to each laboratory by the proficiency testing service FAPAS, they comprised samples containing: low, medium, and high amounts of great crested newt DNA; negative samples (no great crested newt eDNA); and samples containing inhibitors. The latter samples were included to test the laboratories ability to determine samples which are inhibited and thus require dilution 1:2 prior to testing for great crested newt (as per the technical advice note). The outcome of the 2017 proficiency testing scheme suggests that four of the seven laboratories taking part have correctly identified the 'blind' samples (see Table 1). The laboratories were anonymised on the results document so other than ourselves (Lab 002 in this iteration, highlighted in yellow), we do not know which laboratory obtained which results.

Table 1: Tabulated proficiency testing results for all laboratories taking part in the scheme provided by FAPAS.

Lab	Test material A (high level GCN)	Test material B (blank plus inhibitor)	Test material C (medium level GCN)	Test material D (blank plus inhibitor)	Test material E (low level GCN)	Test material F (blank)	Test material G (blank)
001	7/12	0/12 (I)	3/12	0/12 (ND)	0/12	0/12	0/12
002	12/12	0/12 (I)	11/12	0/12 (I)	2/12	0/12	0/12
003	3/12	0/12 (I)	5/12	0/12 (I)	1/12	0/12	0/12
004	10/12	0/12 (I)	11/12	0/12 (I)	1/12	0/12	0/12
005	0/12	0/12 (I)	11/12	0/12 (I)	9/12	7/12	0/12
006	1/12	0/12 (I)	11/12	0/12 (I)	1/12	0/12	0/12
007	12/12	0/12 (I)	4/12	0/12 (I)	0/12	0/12	0/12

I: Inhibition detected

ND: Inhibition not detected

but which contain ultra-pure water (DNA and RNA free water) to monitor for any issues. All controls, extraction blanks and 'blank' samples run this year have been negative as expected illustrating that we have not had any contamination issues in our laboratories. On the other hand, correctly distinguishing 'blind' samples which should be classed as positive is also important as a false-negative result could adversely affect great crested newts, especially if the pond in question is a breeding pond.

Laboratories offering this service must continually monitor and improve their service so that the ecology community as a whole can be confident that this technique is robust and therefore a useful tool for great crested newt presence/absence determination.

It is important for laboratories offering this service to be able to correctly distinguish between positive and negative samples to reduce the risk of false positive and false negative results. Correctly determining 'blind' samples which should be classed as negative is important as a false positive result could indicate potential contamination issues within the laboratory. In addition to the negative controls run on every 96-well plate and extraction blanks, we routinely run 'blank' plates and/or rows on plates which do not contain any sample DNA