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Questions arising from the Report *Investigation of the Performance of Assays for Lyme Disease in Australia* from the National Serology Reference Laboratory

Question 1 – Please provide the comment and advice (including, but not limited to, all documented correspondence) that NRL received from ICPMR and ARRL as stated in Section 3 – Specimens (p9).

Question 2 - Numbers, cross references and calculations. There are errors in the consistency between numbers stated across the NRL report. They should be amended to reflect accurate data, collected, reported and concluded. For example:

- Table 2 notes 53 clinical neg samples were provided by SNP, Figure 16 Reports on 51;
- Table 2 notes 7 negative samples provided by PaLMs, Figure 17 states 5 negative samples, but reports on 7;
- Table 2 notes 26 negative samples provided by IGeneX, Figure 14 reports on 25 negative samples.

Question 3 – Will NRL provide the full data set that underpins the report? If not, why?

There are no evaluable data in this report. For transparency NRL should provide each test result for each specimen, tested with each kit and by each laboratory.

Summary tables which cannot be replicated or interrogated are not useful and may not be reliable given the data errors reported above.

Question 4 - Why does the report not provide data on the sensitivity and specificity of each of the test kits against the organism to which it is targeted? What are the relevant sensitivities and specificities of these tests, as reported by kit manufacturers?

Question 5 – For transparency, the NRL should publish the full data as provided by manufacturers or distributors of the kits used. IFU and Manufacturers disclaimers about their test kits and their interpretive instructions should be published as an Appendix to the study.

Question 6 – The report notes that the ARCBS samples were tested with 5 IA's, and 132 of the samples were tested with IB's also; the report does not provide detailed data by test kit on those samples. Please refer to Q3.

Question 7 – The report notes *"Of the five immunoassay and five immunoblots, three and one respectively were included on the Australian Register of Therapeutic Goods (ARTG)."* Please provide details of the test kits included on the ATRG, pre-July 2017 and post July 2017.

Question 8 – Please indicate which of the IB results were read with scanners and which were read by humans. Please also indicate the method used to read IB's from each of the laboratories who contributed samples that were validated using an IB, or the two tier algorithm.

Question 9 – On the intake of collaborator samples, the Report notes the test kits used by the laboratories for qualifying the samples positive or negative. It also notes the test kits 'used for the past several years'. We know that some labs have changed kits for various reasons, as such the report should clearly state the DATE of the specimen collected and WHICH kit was used for verification of the result by the lab.

Question 10 – The Report recommends (p6) .. *To allow access to a wider range of immunoblots in Australia, without requiring individual laboratories to be the Sponsors, the establishment of a national reference laboratory could be considered. This laboratory could be responsible for evaluating Borrelia IVDs, whether or not they are included on the ARTG. The laboratory could also be responsible nationally for confirmatory testing. Such a laboratory could be an established medical testing laboratory with experience in Lyme disease testing or a laboratory with experience in providing a quality assurance program service.*

ICPMR is a national reference laboratory performing confirmatory assays for Lyme disease, and it is suggested in the report regarding the reason ICPMR did not participate in the study – because they are a reference lab. This recommendation is confusing and requires clarification because a reference laboratory already exists.

Question 11 – The purpose of this study was to understand why serology testing results for Lyme disease in Australia and overseas labs were discordant. Refer CACLD DWG and Senate QoN's.

The conclusions state "*Whilst the tests are relatively under-developed, results reported by NATA accredited laboratories in Australia were consistent with those of other laboratories and tests internationally and there is confidence that active infections with Borrelia burgdorferi are appropriately detected or, alternatively, excluded using these tests in Australia more than 80% of the time".*

This conclusion does not concur with the data reported in the inaccessible tables provided from Figures 4 – 17 and is misleading.

NRL should make available the full data set and excel spreadsheets so the data can be properly interrogated.

Question 12 – According to the inaccessible tables presented from Figures 4 – 17, the report shows a high rate of false negatives among the positive samples as well a significant rate of equivocal results. The Figures provide contrary data to the conclusion that the *'results reported by NATA accredited laboratories in Australia were consistent with those of other laboratories and tests'* is incorrect and misleading.

Making the full data set available is critical. Refer Question 3.

Question 13 – The NRL should publish a table of the interpretive criteria used in this study. It should show the interpretive criteria used by NRL, the manufacturers recommended interpretive criteria by kit and by band for IB's.

Question 14 – The reports executive summary notes that the '*100 specimens provided by PHE were considered the known positive specimen panel in the Project',* yet the data provided on these specimens provides only an 83% avg concordance rate. What is NRL's explanation for the high incidence of false negatives and equivocal results?

Question 15 – According to the report, the *'308 specimens provided by Australian blood donors formed the known negative specimen panel'* . The report then reports that '*Eighty-two specimens had given reactivity at least once in any of the EIAs/immunoassays'.* What is the explanation for the seemingly high (26.6%) false negatives in this sample?

Why were these 82 specimens that provided reactivity in any of the IA's used in the 'known negative' calculations for specificity if they provided a questionable result? Did NRL conduct a two-tier test on them to further validate the specimens, and if so with what kits, and where is that data?

Please provide the full data set on the specimens tested with 1 hour incubation and overnight incubation using Mikrogen test.