Trends in Q Fever EQA Data and Implications for Standardisation

Ashley Prasad[1], Jenny Robson[2], Felicity Rodriguez[1], Meryta May[2], Peter Graham[1]

1 Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP), St Leonards, NSW, Australia

2 Sullivan Nicolaides Pathology, Bowen Hills, QLD, Australia

Introduction

Serology results are used to confirm and monitor Q Fever. However, variation between laboratory methods can present ongoing challenges when interpreting serological results.

Algorithms using combinations such as Q Fever Phase 2 (Q2) and Phase 1 (Q1) IgG and IgM have supported improved diagnostic accuracy and potential staging of infection, distinguishing between acute, past, and evolving chronic Q fever[1]. The uptake of Q2 antigen testing also supports its clinical relevance in diagnosing acute infection[2].

The RCPAQAP offers a Q Fever Serology program with 35 Australian and international laboratories participating. We sought to compare the current Australian Q Fever serology testing profiles and related interpretations using data from the 2024 RCPAQAP Q Fever Serology program.

Method

Four serum samples (x2 negative, 1x acute resolving, 1x evolving chronic) were pre-tested and distributed to twelve Australian laboratories enrolled in the 2024 Q-Fever program. The returned quantitative results and the related qualitative interpretations (Positive/Equivocal/Negative) were reviewed and compared across three methods: enzyme immunoassay (EIA), indirect immunofluorescence assay (IFA), and complement fixation test (CFT).

Results

As shown in Table 1, participating laboratories used a variety of commercial and or in-house kits. These included combinations such as Q2 IgG and IgM by EIA, Q2 IgM by IFA or total antibody detection for both Q1 and Q2 by IFA. IFA is the reference method for the serodiagnosis of Q fever[3]. All returned results were consistent with the pre-testing.

There were no false positives reported across all methods for the **negative** samples.

Positive results were reported for all the IFA methods for the **acute resolving** sample. Q1 IgG titres ranged from 160 to 400, compared to Q2 IgG titres, which were significantly higher (800 to 4096). IgM levels ≥50 were consistent across all methods. Variation in IgA IFA and CFT results was also noted (Figure 2). All five EIA assays returned concordant positive results. It was noted that five laboratories did not test for Q1 IgM in this sample (not shown in Table 1).

The **evolving chronic** sample also returned a wide range of IFA titres (from 1280 to 32768) for Q1 and (256 to 6400) for Q2 IgG (Figure 3). Four EIA and one CFT method reported positive for Q1 IgM and Q2 IgM. There was one discordant EIA/IFU result for this sample.

Discussion and Conclusion

The consensus in negative sample results supports suitable assay specificity.

As expected for the acute resolving sample, within IFA methods, a Q2 > Q1 pattern was observed, and despite titre variability, consistent qualitative interpretations were returned for this high titre sample (Figure 2). The elevated titres of both Q1/Q2 IgM, combined with a lower Q1 IgG response relative to Q2 IgG, support a serological profile consistent with acute or early convalescent Q fever.

All IFA methods for Q1 IgG reported results for the evolving chronic sample that exceeded internationally referenced Duke criteria thresholds for chronic Q fever, i.e Q1 IgG >800 (Minor Dukes), ≥ 1280 (Dutch), and ≥6400 (Major Dukes)[4], as illustrated in Figure 3. Q1 IgG ≥ Q2 IgG titres span more than 16-fold, especially in isotypes with higher endpoint titres. This result profile is consistent with past exposure or possible evolving chronic Q fever infection (Figure 1).

The wide range of reported titres across IFA methods compared to ELISAs in our study is consistent with the WHO/MHRA studies[5]. This underlines the need for ongoing standardisation in result interpretation and reporting. ELISA methods consistently showed lower variability, reinforcing their potential suitability for standardisation across laboratories.

Although this study is limited, there is some evidence, based on the patterns of the returned results (e.g. acute resolving) that recommended testing algorithms are not currently being consistently followed. The results also highlight the need to improve standardisation of the assays to support the result profiles for accurate diagnosis.

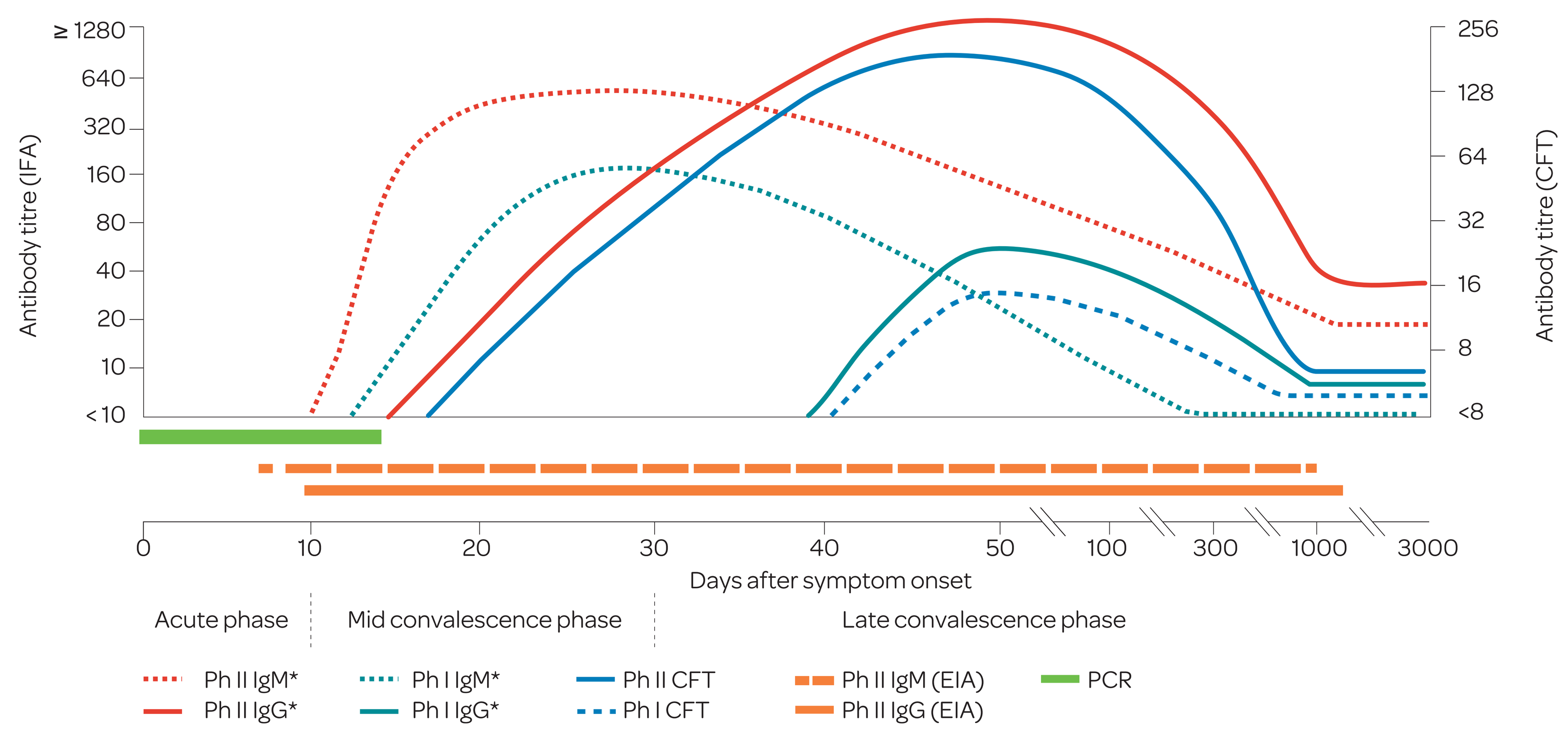

Figure 1 Typical serological responses in Q Fever[1].

Table 1 Overview of assay and antibody targets used by the twelve participating laboratories across the three phases covered by survey samples. The expected testing profiles are based on Duke criteria[1].

Method	No. of Labs	Expected Testing Profiles: Negative	Acute Resolving	Evolving Chronic
		Negative for: Q2 IgG/IgM, Q1 IgG/IgM	Positive for: Q2 IgG/IgM, Q1 IgG/IgM	Positive for: Q2 IgG, Q1 IgG; Negative for: Q1 IgM
Enzyme immunoassay (EIA)	5	All methods reported 'Negative' for Q2 IgG/IgM, Q1 IgG/IgM	All methods reported 'Positive' results for Q1 IgM, Q2 IgM	All methods reported 'Positive' results for Q2 IgG and 'Negative' for Q1 IgM 2 Labs reported discordant Positive/Equivocal results for the same EIA / IFU methods used by the other laboratories
Indirect immunofluorescence assay (IFA)	6			
Complement fixation test (CFT)	1			

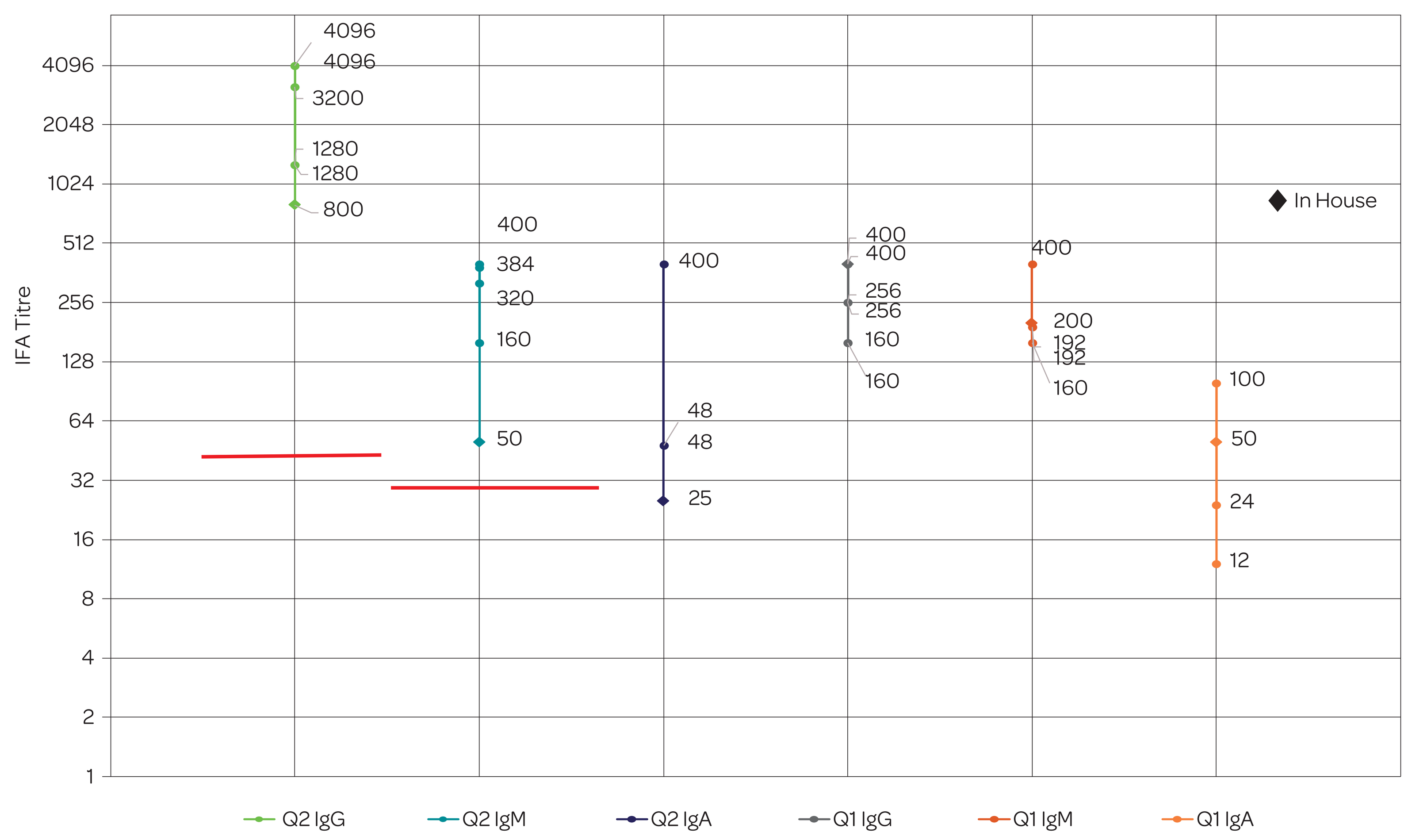

Figure 2 Comparison of IFA titres for the acute resolving sample by phase (Q1, Q2) and isotype (IgG, IgM, IgA). Red lines indicate commonly referenced clinical cut-offs[2].

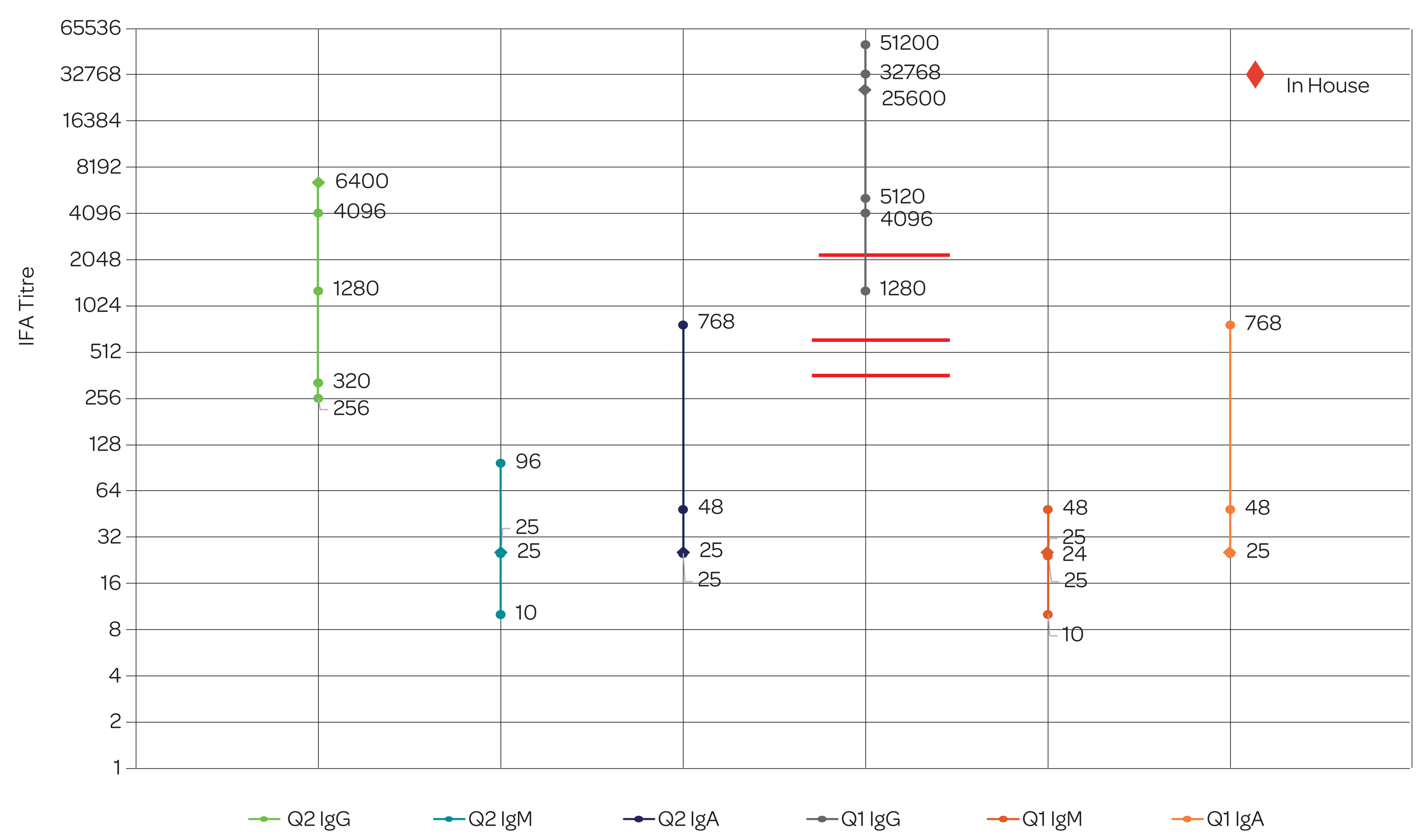

Figure 3 Comparison of IFA titres for the evolving chronic sample by phase (Q1, Q2) and isotype (IgG, IgM, IgA). Red lines indicate Duke criteria thresholds for Q1 IgG[4].

References:

1. NSW Health. Appendix 5: Q fever laboratory result interpretation. In: Q fever control guideline. Sydney: NSW Health; 2019. Available from: https://www.health.nsw.gov.au/Infectious/controlguideline/Pages/qfever-appendix5.aspx
2. Maduranga S, Shrestha LB, Valencia BM, Horton G, Guppy M, Gidding HF, et al. Diagnosing and preventing Q fever in regional New South Wales, Australia—A qualitative exploration of perspectives of general practitioners. *Aust J Rural Health*. 2025. doi:10.1111/ajr.70030.
3. Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. J Clin Microbiology. 1998 Jul;36(7):1823–34.
4. Chu VH, Wang A. Chronic Q fever (including endocarditis) [Internet]. In: Otto CM, Sexton DJ, editors. UpToDate. Baron EL, deputy editor. Waltham (MA): UpToDate; 2024 Sep 24. Available from: https://www.uptodate.com/contents/chronic-q-fever-including-endocarditis
5. World Health Organization. WHO 1st International Standard for anti-Q Fever serum (human). Geneva: World Health Organization; 2023. Report No.: WHO/BS/2023.2456. Available from: who_bs.2023.2456-anti-q-fever-serum.pdf