# Assessment of positivity in immuno-assays with variability in background measurements: a new approach applied to the antibody response to Plasmodium falciparum MSP2

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Measurements of immune responses often exhibit considerable heterogeneity, making it impossible to clearly distinguish responders and nonresponders to particular antigens. Typically, in, for example, enzyme-linked immunosorbent assay (ELISA) procedures, a nonexposed control group is used to assign a cutoff value of positivity, calculated as the mean plus either 2 or 3 standard deviations (S.D.). This can cause extremely biased estimates of response rates when the background is variable, and especially when there is overlap between the distribution of the control levels and that of responders. This problem is compounded when results of assays with different background levels are compared. We illustrate this with hypothetical data sets reflecting frequent patterns seen in laboratory and epidemiological studies. We propose that such data should be analysed by statistical modelling of the ratio of numbers of test samples/control samples as a function of the readout from the assay. Rather than classifying samples dichotomously as negative or positive, this provides estimates of the prevalence of positivity lambda, and the probability, for each sample, that the measured activity is above background. Several statistical methods can provide such estimates. Analyses of simulated data sets using our preferred estimation method [a latent class model (LCM)] demonstrate that this gives more reliable results than the traditional assignment using cutoff values. We have applied this approach to the analysis of ELISA assessments of antibodies against distinct regions of the Plasmodium falciparum merozoite surface protein 2 (MSP2) in human sera from Tanzania.