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Analysis of Liquid Bead Microarray Antibody Assay Data for Epidemiologic Studies of Pathogen-Cancer Associations

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Abstract

Background—Liquid bead microarray antibody (LBMA) assays are used to assess pathogen-cancer associations. However, studies analyze LBMA data differently, limiting comparability.

Methods—We generated 10,000 Monte Carlo-type simulations of log-normal antibody distributions (exposure) with 200 cases and 200 controls (outcome). We estimated type I error rates, statistical power, and bias associated with t-tests, logistic regression with a linear exposure and with the exposure dichotomized at 200 units, 400 units, the mean among controls plus two standard deviations, and the value corresponding to the optimal sensitivity and specificity. We also applied these models, and data visualizations (kernel density plots, receiver operating characteristic (ROC) curves, predicted probability plots, and Q-Q plots), to two empirical datasets to assess the consistency of the exposure-outcome relationship.
Results—All strategies had acceptable type I error rates (0.03 ≤ P ≤ 0.048), except for the dichotomization according to optimal sensitivity and specificity, which had a type I error rate of 0.27. Among the remaining methods, logistic regression with a linear predictor (Power=1.00) and t-tests (Power=1.00) had the highest power to detect a mean difference of 1.0 MFI (median fluorescence intensity) on the log scale and were unbiased. Dichotomization methods upwardly biased the risk estimates.

Conclusion—These results indicate that logistic regression with linear predictors and unpaired t-tests are superior to logistic regression with dichotomized predictors for assessing disease associations with LBMA data. Logistic regression with continuous linear predictors and t-tests are preferable to commonly used LBMA dichotomization methods.

Keywords
liquid bead microarray antibody assay; median fluorescence intensity; cut-point; dichotomization; visualization

1. Introduction
Serologic antibody assays are important tools for investigating associations of infectious diseases with acute and chronic conditions in epidemiologic studies. Since the start of the third millennium (Jones et al., 2002), liquid bead microarray antibody (LBMA) assays have been used to screen for antibodies to pathogens that may be associated with human cancers (Burnett-Hartman et al., 2011; Carter et al., 2009; Clifford et al., 2007; Gu et al., 2008; Michael et al., 2008; Rowhani-Rahbar et al., 2009; Waterboer et al., 2005). One such pathogen, human papillomavirus (HPV), is an established cause of cervical (Muñoz et al., 1992), other anogenital (Frisch et al., 1997), and oropharyngeal (Gillison et al., 2000) cancers. High-risk oncogenic HPV types are sexually transmitted and have been the subject of numerous studies utilizing LBMA. The association of Merkel cell polyomavirus (MCPyV) with Merkel cell carcinoma (Feng et al., 2008), an aggressive skin cancer, has also been studied using this technology (Carter et al., 2009; Paulson et al., 2010).

LBMA assays test for serum antibodies by incubating sera with fluorescently labeled microspheres that are bound to antigens of interest. Biotinylated secondary antibodies are then bound to the primary antibodies and are fluorescently labeled with streptavidin-phycoerythrin. Flow cytometry is used to generate the median fluorescence intensity (MFI), a continuous measure of the amount of phycoerythrin that is bound to the beads. Therefore, the MFI is a surrogate measure of the strength of an antibody response to a particular antigen. The core advantage of LBMA is its multiplexing capability, which allows for screening of hundreds of distinct antibodies at once, far more than with enzyme-linked immunosorbent assays (ELISA). LBMA multiplexing saves time, conserves sera for future studies, and is high-throughput, with the ability to analyze up to 1,000 specimens daily. Yet, the lack of a standard method for analyzing LBMA data limits the comparability of results across studies of the same agents.

As with ELISA (Frey et al., 1998), the absence of available standards and researchers’ desire for binary immune status indicators (i.e., seropositive vs. seronegative) have led to defining
MFI cut-points in a variety of ways in studies utilizing LMBA. For example, previous publications on human papillomavirus (HPV) defined HPV-related MFI cut-points in at least four different ways: the mean value in healthy blood donors plus three standard deviations (SD) (Waterboer et al., 2005), five SD above the mean of the sampled distribution (after removing outliers) (Clifford et al., 2007), the mean values among virgins plus two SDs (Rowhani-Rahbar et al., 2009), and the mean values among healthy blood donors with only one lifetime sex partner plus two SDs (Burnett-Hartman et al., 2011). Studies that used standard deviations to define cut-points likely assumed a normal distribution of MFI among unexposed individuals. This would result in approximately 95.4% or 99.7% of uninfected persons falling within two or three SD, respectively. However, the distributions are not necessarily normal and these methods would misclassify a percentage of uninfected participants as infected. In studies of sexually active individuals that lack samples from persons with low exposure to sexually transmitted HPV (i.e., virgins), MFI cut-points have been determined using receiver operating characteristic (ROC) curves (Gu et al., 2008), arbitrary a priori thresholds (Michael et al., 2008), and the upper quartile of MFI among control participants (Carter et al., 2009). This broad variety of methods used hinders comparisons of results across studies.

Even if a standard method for dichotomizing LBMA data existed, doing so may be problematic. Dichotomizing continuous predictors can reduce statistical power by up to a third or half (Lagakos, 1988; Ragland, 1992; Selvin, 1987), inflating the needed sample sizes and associated costs. In addition, dichotomization may conceal more complex exposure-outcome relationships (Breslow and Day, 1980) and may influence the estimated measure of association by introducing bias (Wartenberg and Northridge, 1991). In response, some have sought to avoid the dichotomization dilemma by testing for differences between mean MFI using one-way analysis of variance (ANOVA) or t-tests (Gu et al., 2008). Others suggested modeling continuous data, while freeing it from the constraints of linear assumptions. For example, though it does not provide a concise summary test statistic, cubic spline regression allows for close approximation of almost any smooth curve and can provide risk estimates over the full range of the continuous predictor (Greenland, 1995). Similarly, quantile-quantile (Q-Q) plots, which depict the cumulative distribution of cases and controls as well as the odds ratio (OR) for all levels of a continuous predictor, have been suggested as an exploratory tool (Wartenberg and Northridge, 1991).

Our primary objective was to identify the most statistically efficient and unbiased method(s) for detecting associations between LBMA antibody measures (MFI) and disease status in epidemiologic studies of virus-cancer associations. Our secondary objective was to compare the utility of different data visualizations of MFI and disease status as exploratory tools. We used both simulated and empirical datasets of natural HPV and MCPyV infection as our motivating examples. We pursued both objectives with the goal of encouraging researchers working in this field to adopt standards that will improve comparability across studies.
2. Materials and Methods

2.1 Data Sources

To estimate the statistical power (1 minus the false-negative proportion), type I error (the false-positive proportion), and bias resulting from different analytic methods, we generated 10,000 Monte Carlo-type simulated datasets per analysis. Each dataset contained 200 controls and 200 cases randomly selected from natural log (ln) normal distributions of MFI. We set the parent distribution of the controls to have a mean of 3 and a standard deviation (SD) of 1.6. The parent distributions of the cases had set means of 3, 4, 5, 6, 7, 8, or 9 and a SD of 1.6. The selected distributions represent positive associations over the linear range of the assay and within the range of MFI data that are often observed in studies of natural HPV infection using LBMA (Carter et al., 2009; Michael et al., 2008; Waterboer et al., 2009, 2005). For example, lnMFI of 3 corresponds with MFI=20, which is low, and lnMFI of 9 corresponds with MFI=8,103, which is extremely high.

We also investigated potential differences in measures of the association between MFI and disease status in empirical data. We first selected a dataset with a published null association to assess whether using other analytic methods would have identified a positive association. Specifically, we utilized a dataset from a case-control study of HPV antibodies in association with colorectal hyperplastic polyps in men from the Minnesota Cancer Prevention Research Unit Polyp Study (Burnett-Hartman et al., 2012). Using LBMA to test plasma from 97 cases and 184 controls and a cut-point of greater than 400 MFI for seropositivity, the authors found no significant association between any HPV antibodies and hyperplastic polyps. The crude OR for HPV-16 L1 antibodies was 0.62 (95% confidence interval (CI): 0.16–2.35), and no rationale for the pre-specified cut-point was provided in the publication. We reanalyzed the HPV-16 antibody data from that study because it is the HPV type most commonly associated with cancer (Muñoz et al., 2004; Schiffman et al., 2007) and is commonly assessed in LBMA-based serologic studies of HPV.

In addition, we reanalyzed a dataset with a published positive association to assess the consistency of that association when using alternative analytic methods. The dataset was from a case-control study of Merkel cell polyomavirus antibodies in association with Merkel cell carcinoma (Paulson et al., 2010). The cases (n=139) were Merkel cell carcinoma patients from the Merkel Cell Carcinoma Repository of Patient Data and Specimens at the Fred Hutchinson Cancer Research Center. The population-based controls (n=530) were recruited from Western Washington via random digit dialing and were frequency matched on age and sex. Using cut-points of three SDs above the mean of controls, the authors reported an OR of 16.9 (95% CI: 7.8–36.7) for Merkel cell polyomavirus large t-antigen seropositivity.

2.2 Analysis Plan

We evaluated the simulated datasets with case-control status as the outcome and MFI as the predictor. We tested associations using three types of analytic techniques: (a) t-tests; (b) logistic regression with a linear predictor; and (c) logistic regression with predictors dichotomized according to four methods described in the literature. Those methods defined
cut-points as: 200 or 400 MFI (Burnett-Hartman et al., 2012; Michael et al., 2008) determined a priori; the mean MFI among controls plus two SDs (Burnett-Hartman et al., 2011); and the optimal value based upon ROC curve analysis (Gu et al., 2008). Because the last method was not described in detail in previous publications, we used the MFI cut-point corresponding to the maximum J-statistic (sensitivity + specificity − 1), which is also referred to as Youden’s index (Youden, 1950). The J-statistic, which is distinct from the J-test, is a summary measure of diagnostic test performance that ranges from zero (useless) to one (perfect) (Youden, 1950). For each analytic method, we calculated statistical power as the ratio of the number of tests with $P$-values <0.05 compared to the total number of tests (10,000). Type I error calculations were the same as those for power, but were limited to situations where the true mean difference was zero.

We also estimated bias using the simulated datasets with a true mean difference of zero. The bias for the logistic regression models was estimated by calculating the mean OR minus 1. Similarly, we estimated the bias for the t-tests by calculating the mean of the mean differences.

We then analyzed the empirical datasets using: logistic regression with a linear predictor; logistic regressions with each of the four types of dichotomous predictors; and an unpaired t-test. Logistic regression ORs for the Merkel cell data were adjusted for age and sex, the frequency matching variables. We compared the $P$-value, magnitude of the association, and the direction of the association.

We also compared four different methods of plotting the relationship between continuous MFI and disease status. First, we generated kernel density plots of the distribution of lnMFI among the cases and controls. Second, following an example in the literature (Gu et al., 2008), we performed nonparametric ROC regression and plotted the results as an ROC curve, with the corresponding area under the curve (AUC). We overlaid lines on the ROC curve highlighting the optimal cut-point based upon the maximum J-statistic described above. Third, we created a graph of the predicted probability of being a case (y-axis) in relation to lnMFI (x-axis), based upon a logistic regression model using a restricted cubic spline of MFI with three knots as the predictor (Buis, 2009). Fourth, we generated Q-Q plots with the empirical cumulative distribution function for controls and cases on the x- and y-axes, respectively (Wartenberg and Northridge, 1991). We superimposed the Q-Q plot data on constant OR curves of 100, 20, 10, 5, 4, 3, 2, 1, .5, .33, .25, .20, .10, .05, and .01 to facilitate visual estimation of the OR for any lnMFI value.

We considered $P$-values less than 0.05 to be statistically significant and conducted all analyses using Stata/SE 13.1 (StataCorp, College Station, TX). The Institutional Review Board of the Fred Hutchinson Cancer Research Center approved the secondary analysis of the Minnesota Cancer Prevention Research Unit Polyp Study data. The University of Washington Human Subjects Division determined that the secondary analysis of Merkel Cell Carcinoma Repository of Patient Data and Specimens data did not meet the federal regulatory definition of “human subjects research” under 45 CFR 46.102(f).
3. Results

3.1 Simulation assessment of type I error, power, and bias

In analyses of the simulated data, the type I error using the J-statistic was inflated ($P=0.27$), but was reasonable for all other methods ($0.03 \leq P \leq 0.048$) (Table 1). For a true mean difference of 1 lnMFI, logistic regression with continuous MFI, logistic regression with dichotomization using the maximum J-statistic, and the unpaired t-test had the highest power ($Power=1.00$). Power was lower for logistic regression with dichotomization at 200 MFI ($Power=0.97$), 400 MFI ($Power=0.86$), and the mean of the controls plus two SD ($Power=0.76$).

Logistic regression with a linear predictor and unpaired t-tests generated no bias (data not shown). However, dichotomization at 200 MFI biased the OR upward by 8%; dichotomization at 400 MFI biased the OR upward by 24%; dichotomization at the mean of the controls plus two SD biased the OR upward by 29%; and dichotomization based upon the J-statistic biased the OR upward by 62%.

Kernel density, ROC, predicted probability, and Q-Q plots were created for mean differences of 0, 1, 2, and 3 and are available as Supplementary Figures S1, S2, S3, and S4, respectively. Figure S1 shows completely overlapping kernel density plots, an ROC curve with an AUC of 0.50, a predicted probability graph centered on 0.5 over the full range of lnMFI, and a Q-Q plot with the data plotted along the null referent (OR=1). Figure S2 shows a right shift of the kernel density plot toward higher MFI, an increased AUC (0.67), a slightly sigmoidal diagonal on the predicted probability graph, and a slight downward curve on the Q-Q plot. Figure S3 continues the trends seen in Figure S2, with a greater shifting of the kernel density plot, an increase in the AUC (0.81), a steeper sigmoidal predicted probability graph, and a deeper bend in the Q-Q plot. Figure S4 is similar to S3, but with more pronounced shifting of the kernel density plots, an increased AUC (0.908), an even steeper sigmoidal predicted probability plot, and an even deeper bend in the Q-Q plot.

3.2 Empirical assessment of association with the outcome

In logistic regression analyses of the Minnesota Cancer Prevention Research Unit Polyp Study data, the ORs were not statistically significant ($P>0.05$), regardless of the MFI cut-point used (Table 2). However, there was variation in the estimated direction of the association. Modeling MFI as a continuous linear predictor yielded no association (OR=1.00, 95 CI: 0.87–1.15). In contrast, cut-points at 200 MFI (OR=0.76, 95% CI: 0.31–1.91), 400 MFI (OR=0.62, 95% CI: 0.16–2.35), and the mean among controls plus two SD (OR=0.75, 95% CI: 0.14–3.96) generated ORs less than one, whereas a cut-point based on the maximum J-statistic yielded an OR greater than one (OR=1.59, 95% CI: 0.93–2.72). The t-test produced no evidence of an association ($P=0.99$) and a mean difference of 0. We successfully replicated the crude OR reported in the original publication (OR=0.62, 95% CI: 0.16–2.35) (Burnett-Hartman et al., 2012).

In multivariate logistic regression, adjusting for age and sex, the ORs for Merkel cell polyomavirus antibody data were statistically significant ($P<0.001$), regardless of the MFI cut-point used (Table 3). However, there were differences in the estimated strengths of the
association. Among dichotomizing methods, splitting the data at the mean among controls plus two SD generated the highest OR (9.92, 95% CI: 6.02–16.33) and a cut-point of 200 MFI generated the lowest OR (7.7, 95% CI: 5.0–12.0). The t-test provided evidence of an association (P<0.001) with a mean difference of 2.44. We successfully replicated the adjusted OR reported in the original publication (OR=16.92, 95% CI: 7.80–36.39) (Paulson et al., 2010).

The kernel density plot of the Minnesota data shows overlapping log-normal curves for cases and controls (Figure 1). The area under the ROC curve is 0.509, with an optimal cut-point of 2.9 lnMFI, based upon the maximum J-statistic. The predicted probability graph is essentially flat, with no evidence of an association. The entire lnMFI line on the Q-Q plot is close to the null referent curve (OR=1), with no evidence for an association with increasing MFI.

In the kernel density plot of the Merkel cell polyomavirus large t-antigen data, a distinct right shift of the peak for cases compared to controls can be seen (Figure 2). The area under the ROC curve is 0.678, with an optimal cut-point of 5.3 lnMFI, based upon the maximum J-statistic. The predicted probability graph is sigmoidal, with a rapid increase in the likelihood of case status for lnMFI>5. The Q-Q plot shows evidence of an association with case status for lnMFI>5.

4. Discussion

Based upon our simulated data, logistic regression with continuous linear MFI and unpaired t-tests provided the best, and nearly identical, combinations of high statistical power, reasonable type I error, and unbiased estimates. We anticipated this result based on the literature cited in the introduction above. Application of these methods to empirical data also demonstrated the similarity between these two analytic methods compared to the other four that we assessed.

The four data visualizations were consistent in their depiction of the association between lnMFI and disease status in the empirical datasets. However, unlike the other three graphs, the ROC curve lacks information regarding the data density informing the structure of the curve. Of the remaining three, a benefit of the predicted probability (restricted cubic spline) plot is the facility with which researchers can adjust for potential confounding in the underlying logistic regression model. Regardless of the visualization selected, if researchers are interested in formal hypothesis testing, rather than simply describing the data, considerations should be given to the problem of multiple comparisons (Wartenberg and Northridge, 1991). Specifically, when researchers use graphs to inform the selection of a cut-point, they have in fact already visually assessed and discarded a series of potential cut-points to select the one they believe is most promising in light of their hypothesis. This is a violation of core assumptions underlying a priori hypothesis testing (Wartenberg and Northridge, 1991) and leads to inflation of the type I error rate. Therefore, risk estimates based on cut-points selected in this manner are best reported as exploratory findings and should be presented alongside estimates based on a range of alternative cut-points. P-values should not be reported. Those desiring to use visualizations to inform selection of cut-points...
for causal inference may consider data splitting (Dahl et al., 2008). Partitioning the data randomly into two parts would allow the investigator to develop a hypothesis based on visual exploratory analyses of the first portion of the data while preserving the second portion of the data for hypothesis testing.

The literature demonstrates a strong preference among researchers for dichotomizing LBMA-based HPV antibody data. In light of our findings, we recommend that researchers consider other analytic methods. Analyses using unpaired t-tests and logistic regression with linear continuous MFI are two simpler and yet statistically more powerful alternatives. However, there are valid concerns regarding the use of linear models. A linear model may obscure threshold effects or other non-linear relationships due to its assumption that a 1-unit change at low MFI values has the same association with disease risk as a 1-unit change at high MFI values. The validity of these assumptions could be assessed using predicted probability plots in an exploratory sub-dataset as mentioned above. Alternatively, analyzing the data by quartiles would allow the assessment of both threshold effects and dose response relationships at the same time. The use of non-dichotomized predictors also makes interpretation of causal inference challenging. For example, the meaning of “HPV seropositivity increases the odds of developing a cancer two-fold” is more straightforward than “a 1 unit increase in HPV lnMFI increases the odds of developing a cancer two-fold.” For this reason, linear models are probably best suited for research attempting to establish, rather than quantify, a correlation between an infection and cancer status.

In conclusion, based upon type I error, statistical power, and bias, both logistic regression with continuous linear MFI and unpaired t-tests were superior to logistic regression with dichotomized MFI. Data splitting should be considered if visualizations are to inform selection of cut-points for causal inference.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**Abbreviations**

ANOVA analysis of variance  
AUC area under the curve  
CI confidence interval
ELISA  enzyme-linked immunosorbent assays
HPV  human papillomavirus
LBMA  liquid bead microarray antibody
ln  natural log
MCPyV  Merkel cell polyomavirus
MFI  median fluorescence intensity
OR  odds ratio
Q-Q  quantile-quantile
ROC  receiver operating characteristic
SD  standard deviation

References

Buis ML. POSTRCSPLINE: Stata module containing post-estimation commands for models using a restricted cubic spline. 2009
Greenland S. Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. 

Gu A, Xie Y, Mo H, Jia W, Li MY, Li M, Chen L, Feng Q, Liu Q, Qian C, Zeng Y. Antibodies against 
Epstein-Barr virus gp78 antigen: a novel marker for serological diagnosis of nasopharyngeal 
carcinoma detected by xMAP technology. J Gen Virol. 2008; 89:1152–8.10.1099/vir.0.83686-0
[PubMed: 18420792]

Jones LP, Zheng H, Karron RA, Peret TCT, Tsou C, Anderson LJ. Multiplex assay for detection of 
strain-specific antibodies against the two variable regions of the G protein of respiratory syncytial 

Lagakos SW. Effects of mismodelling and mismeasuring explanatory variables on tests of their 

Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H, Bravo IG, Schlehofer J, Gärtner 
BC, Pawlita M. Seroprevalence of 34 human papillomavirus types in the German general 

Muñoz N, Bosch FX, Castellsagué X, Díaz M, de Sanjose S, Hammouda D, Shah KV, Meijer CJLM. 
Against which human papillomavirus types shall we vaccinate and screen? The international 

Muñoz N, Bosch FX, de Sanjose S, Tafur L, Izarzugaza I, Gili M, Viladiu P, Navarro C, Cartos C, 
Ascunce N. The causal link between human papillomavirus and invasive cervical cancer: a 
[PubMed: 1330933]

Paulson KG, Carter JJ, Johnson LG, Cahill KW, Iyer JG, Schrama D, Becker JC, Madeleine MM, 
Nghiem P, Galloway DA. Antibodies to merkel cell polyomavirus T antigen oncoproteins reflect 
tumor burden in merkel cell carcinoma patients. Cancer Res. 2010; 70:8388– 
97.10.1158/0008-5472.CAN-10-2128 [PubMed: 20959478]

Ragland DR. Dichotomizing continuous outcome variables: dependence of the magnitude of 
1391136]

Rowhani-Rahbar A, Carter JJ, Hawes SE, Hughes JP, Weiss NS, Galloway DA, Koutsky LA. 
Antibody responses in oral fluid after administration of prophylactic human papillomavirus 

Schiffman M, Castle PE, Jaromino J, Rodriguez AC, Wacholder S. Human papillomavirus and 
[PubMed: 17826171]

[PubMed: 3653356]


Waterboer T, Neale R, Michael KM, Sehr P, de Koning MNC, Weissenborn SJ, Sampogna F, Abeni 
D, Green AC, Bouwes Bavinck JN, Pawlita M. Antibody responses to 26 skin human 
papillomavirus types in the Netherlands, Italy and Australia. J Gen Virol. 2009; 90:1986– 
98.10.1099/vir.0.010637-0 [PubMed: 19386782]

Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, Templin MF, Pawlita M. 
Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion 

### Highlights

- Liquid bead microarray antibody assay data has been analyzed in different ways.
- We compared analysis methods with regard to statistical efficiency and bias.
- Logistic regression with linear predictors and t-tests were unbiased and efficient.
Figure 1.
Graphical representations of the association between HPV-16 L1 MFI and hyperplastic polyps in the Minnesota Cancer Prevention Research Unit Polyp Study.

Top left. The kernel density plot illustrates the probability density function of log-transformed MFI curves for cases and controls. The tick marks represent the density of the data informing the curves. Top right. The ROC curve has reference lines noting the sensitivity and 1-specificity corresponding to lnMFI 2.9, the optimal cut-point based on the maximum J-Statistic. Bottom left. A predicted probability plot based on a restricted cubic spline of MFI, with three knots. The dashed lines outline the 95% confidence interval. Tick marks depict the density of the data informing the curve. The horizontal line is the reference for no association. (#cases/(#cases+#controls)). Bottom right. The Q-Q plot depicts the cumulative distribution function for cases (y-axis) and controls (x-axis) in association with varying lnMFI. The heavy curve represents lnMFI values, with tick marks noting one-unit increments. The distance between tick marks is proportional to the amount of MFI data within that range and the light curves are constant odds ratios.
Figure 2.
Graphical representations of the association between Merkel cell polyomavirus large t-antigen MFI and Merkel cell carcinoma in the Merkel Cell Carcinoma Repository of Patient Data and Specimens, Fred Hutchinson Cancer Research Center.
Top left. The kernel density plot illustrates the probability density function of log-transformed MFI curves for cases and controls. The tick marks represent the density of the data informing the curves. Top right. The ROC curve has reference lines noting the sensitivity and 1-specificity corresponding to lnMFI 5.3, the optimal cut-point based on the maximum J-Statistic. Bottom left. A predicted probability plot based on a restricted cubic spline of MFI, with three knots. The dashed lines outline the 95% confidence interval. Tick marks depict the density of the data informing the curve. The horizontal line is the reference for no association. (#cases/(#cases+#controls)). Bottom right. The Q-Q plot depicts the cumulative distribution function for cases (y-axis) and controls (x-axis) in association with varying lnMFI. The heavy curve represents lnMFI values, with tick marks noting one-unit increments. The distance between tick marks is proportional to the amount of MFI data within that range and the light curves are constant odds ratios.
Table 1

Estimated type 1 error rate and power to detect differences in MFI between cases and controls using logistic regression and an unpaired t-test in simulated datasets.\textsuperscript{a}

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<th>400 MFI\textsuperscript{e}</th>
<th>mean + 2 SD\textsuperscript{f}</th>
<th>J-Statistic\textsuperscript{g}</th>
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<td>0.762</td>
<td>1.000</td>
</tr>
<tr>
<td>1.25</td>
<td>1.000</td>
<td>0.999</td>
<td>0.983</td>
<td>0.934</td>
<td>1.000</td>
</tr>
<tr>
<td>1.50</td>
<td>1.000</td>
<td>1.000</td>
<td>0.999</td>
<td>0.989</td>
<td>1.000</td>
</tr>
<tr>
<td>1.75</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.999</td>
<td>1.000</td>
</tr>
<tr>
<td>2.00</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

\textsuperscript{a}10,000 simulations of 200 controls (mean=3, SD=1.6) and 200 cases (mean=3 + mean difference, SD=1.6).

\textsuperscript{b}MFI is median fluorescence intensity, a measure of the strength of an antibody response.

\textsuperscript{c}Linear continuous form of lnMFI.

\textsuperscript{d}Less than or equal to 200 MFI was considered unexposed.

\textsuperscript{e}Less than or equal to 400 MFI was considered unexposed.

\textsuperscript{f}Less than or equal to the mean lnMFI of the controls plus two standard deviations was considered unexposed.

\textsuperscript{g}Less than the lnMFI corresponding to the maximum J-statistic (Sensitivity + Specificity – 1) was considered unexposed.

\textsuperscript{h}This row, where the null hypothesis is true, estimates the type I error rather than power.
Table 2

The association of HPV 16 L1 MFI\textsuperscript{a} with hyperplastic polyps in the Minnesota Cancer Prevention Research Unit Polyp Study\textsuperscript{b}.

<table>
<thead>
<tr>
<th>MFI\textsuperscript{a} cut-point</th>
<th>OR</th>
<th>Mean Difference</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (continuous)\textsuperscript{c}</td>
<td>1.00</td>
<td>0.87–1.15</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>200 MFI\textsuperscript{d}</td>
<td>0.76</td>
<td>0.31–1.91</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>400 MFI\textsuperscript{e}</td>
<td>0.62</td>
<td>0.16–2.35</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Mean among controls + 2 SD\textsuperscript{f}</td>
<td>0.75</td>
<td>0.14–3.96</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>J-Statistic\textsuperscript{g}</td>
<td>1.59</td>
<td>0.93–2.72</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>t-test</td>
<td>0.00</td>
<td>−0.44–0.45</td>
<td>0.99</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}MFI is median fluorescence intensity, a measure of the strength of an antibody response.

\textsuperscript{b}Cancer Epidemiol Biomarkers Prev 2012;21:1599–601.

\textsuperscript{c}OR is per 1 unit change in lnMFI.

\textsuperscript{d}Less than or equal to 200 MFI was considered unexposed.

\textsuperscript{e}Less than or equal to 400 MFI was considered unexposed.

\textsuperscript{f}Less than or equal to the mean lnMFI of the controls plus two standard deviations was considered unexposed.

\textsuperscript{g}Less than the lnMFI corresponding to the maximum J-statistic (Sensitivity + Specificity − 1) was considered unexposed.
Table 3

The association of Merkel cell polyomavirus large t-antigen MFI\(^a\) with Merkel cell carcinoma in the Merkel Cell Carcinoma Repository of Patient Data and Specimens, Fred Hutchinson Cancer Research Center\(^b\).

<table>
<thead>
<tr>
<th>MFI(^a) cut-point</th>
<th>OR(^c)</th>
<th>Mean Difference</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (continuous)(^d)</td>
<td>1.30</td>
<td>1.22–1.38</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>200 MFI(^e)</td>
<td>7.72</td>
<td>4.98–11.97</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>400 MFI(^f)</td>
<td>9.01</td>
<td>5.62–14.46</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Mean among controls + 2 SD(^g)</td>
<td>9.92</td>
<td>6.02–16.33</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>J-Statistic(^h)</td>
<td>8.25</td>
<td>5.33–12.77</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>t-test</td>
<td>2.44</td>
<td>2.08–2.79</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) MFI is median fluorescence intensity, a measure of the strength of an antibody response.

\(^b\) Cancer Res; 70(21) November 1, 2010.

\(^c\) Adjusted for age and sex.

\(^d\) OR is per 1 unit change in lnMFI.

\(^e\) Less than or equal to 200 MFI was considered unexposed.

\(^f\) Less than or equal to 400 MFI was considered unexposed.

\(^g\) Less than or equal to the mean lnMFI of the controls plus two standard deviations was considered unexposed.

\(^h\) Less than the lnMFI corresponding to the maximum J-statistic (Sensitivity + Specificity – 1) was considered unexposed.