Supplementary Information (SI) for Analytical Methods.

ESI document S1 for Schuster-Little et al.:"Immunoaffinity-free chromatographic purification of ovarian cancer biomarker CA125 (MUC16) from blood serum enables mass spectrometry characterization"

Description of error propagation methods

The measured values from CA125 ELISA and protein BCA assays are absorbances. From these absorbance values, concentrations are inferred using a quadratic polynomial to fit the calibration data. Uncertainties in these concentrations were determined from average absorbance values using a method found in the Engineering Statistics Handbook published by the National Institutes of Standards and Technology (https://www.itl.nist.gov/div898/handbook/mpc/section3/mpc3671.htm, accessed 6/19/2024). Specifically, for a polynomial function of the form

$$Y = a + bX + cX^2 \qquad \text{(Equation 1)}$$

the quadratic formula can be written

$$X' = \frac{-b \pm \sqrt{b^2 - 4c(a - Y')}}{2a} \qquad \text{(Equation 2)}$$

where X' is a calibrated value (concentration in this case) and Y' is a measured value (absorbance in this case). The uncertainty in X' (denoted u) is calculated from partial derivatives and the variance (u^2) as follows:

$$\frac{\partial X'}{\partial Y'} = \frac{1}{\sqrt{b^2 - 4c\,(a - Y')}} \qquad \text{(Equation 3)}$$

$$\frac{\partial X'}{\partial a'} = \frac{-1}{\sqrt{b^2 - 4c\,(a - Y')}} \qquad \text{(Equation 4)}$$

$$\frac{\partial X'}{\partial b} = \frac{-1 + \dfrac{b}{\sqrt{b^2 - 4c\,(a - Y')}}}{2c} \qquad \text{(Equation 5)}$$

$$\frac{\partial X'}{\partial c} = \frac{-a + Y'}{c\sqrt{b^2 - 4c\,(a - Y')}} - \frac{-b + \sqrt{b^2 - 4c\,(a - Y')}}{2c} \qquad \text{(Equation 6)}$$

$$u^2 = \left(\frac{\partial X'}{\partial Y'}\right)^2 (s_y)^2 + \left(\frac{\partial X'}{\partial a'}\right)^2 (s_a)^2 + \left(\frac{\partial X'}{\partial b'}\right)^2 (s_b)^2 + \left(\frac{\partial X'}{\partial c'}\right)^2 (s_c)^2 \qquad \text{(Equation 7)}$$

$$u = \sqrt{\left(\frac{\partial X'}{\partial Y'}\right)^2 (s_y)^2 + \left(\frac{\partial X'}{\partial a'}\right)^2 (s_a)^2 + \left(\frac{\partial X'}{\partial b'}\right)^2 (s_b)^2 + \left(\frac{\partial X'}{\partial c'}\right)^2 (s_c)^2} \quad \text{(Equation 8)}$$