

## Central Lancashire Online Knowledge (CLoK)

Title	Investigating centrifugal filtration of serum-based FTIR spectroscopy for the stratification of brain tumours
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Creators	Theakstone, Ashton, Brennan, Paul, Jenkinson, Michael, Goodacre, Royston and Baker, Matthew

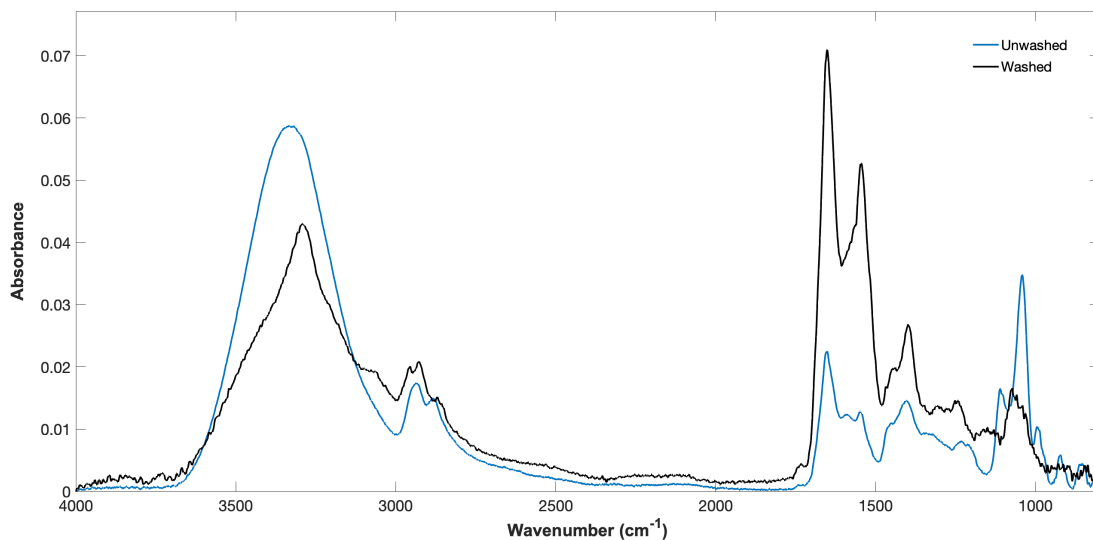
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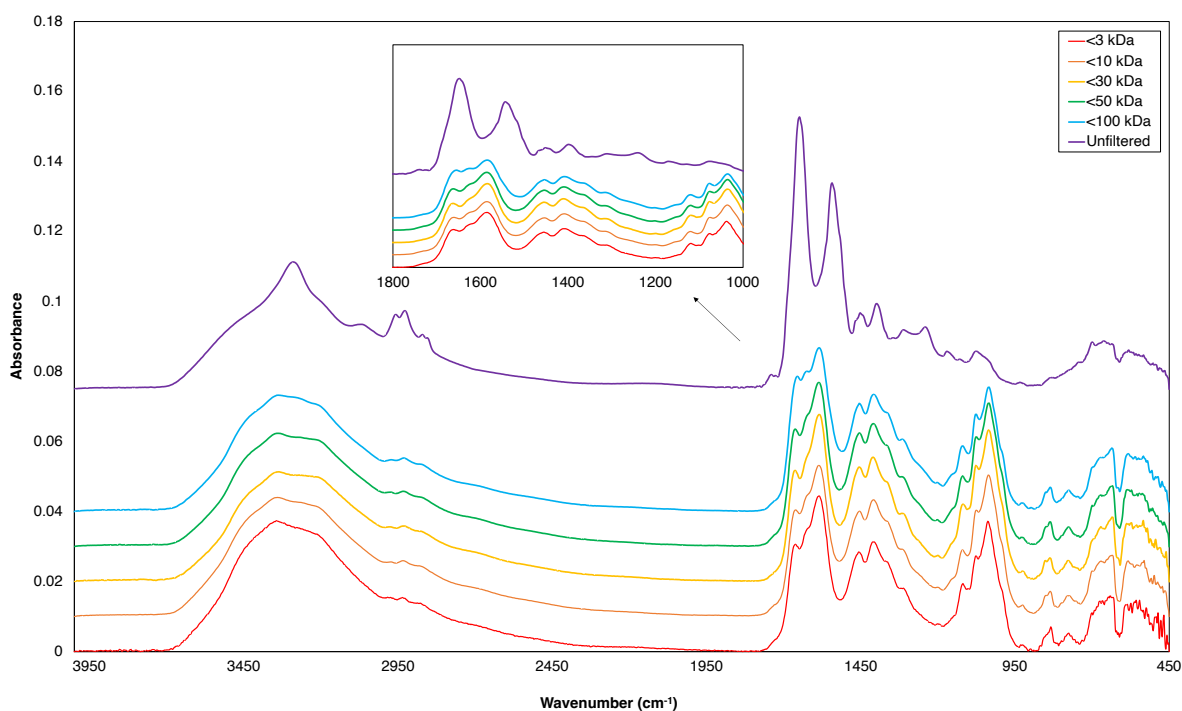
# Investigating centrifugal filtration of serum-based FTIR spectroscopy for the stratification of brain tumours

## SUPPORTING INFORMATION



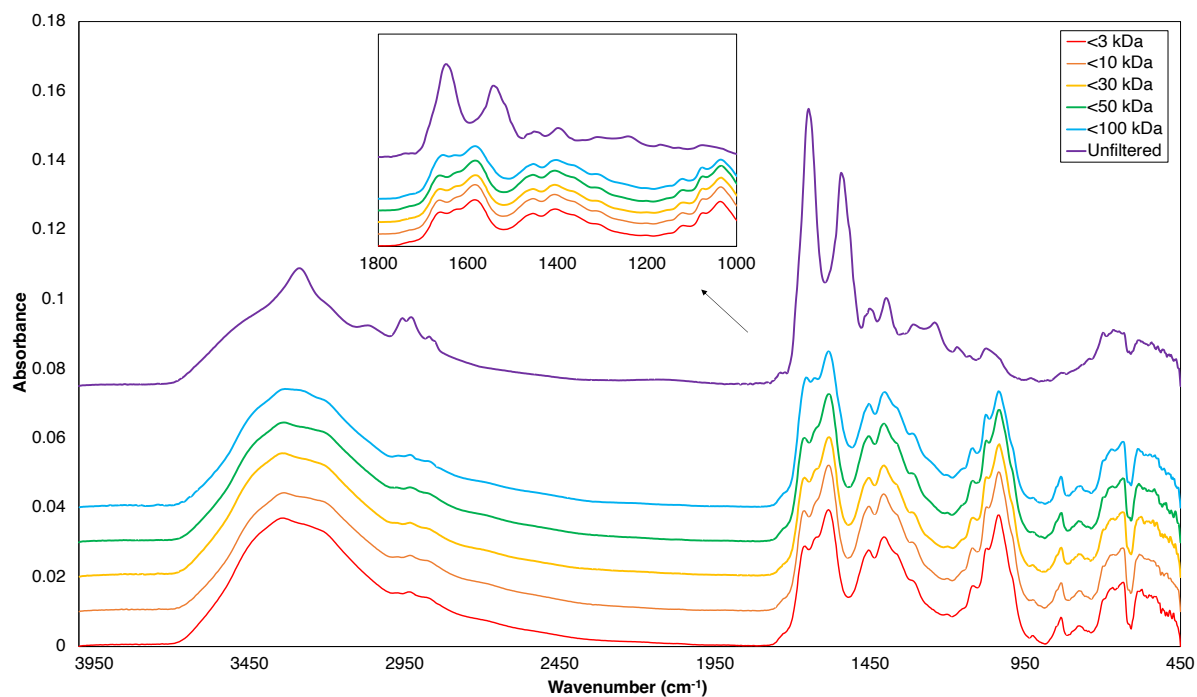
**Fig S1. Example spectra of a non-cancer patient.**

Serum was fractionated through two 100 kDa molecular weight cut-off filters. The blue line represents the spectral profile with an unwashed filter and the black line is a washed filter.



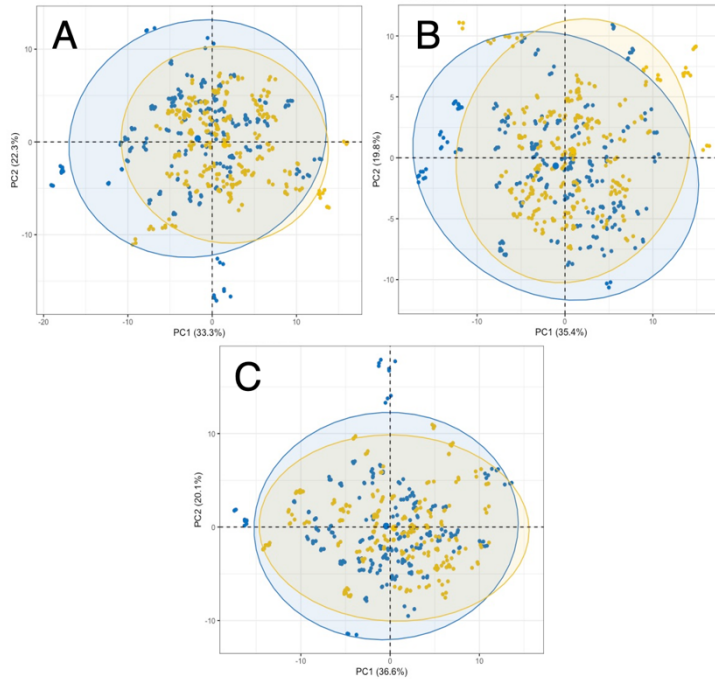
**Fig S2. Patient serum spectra including unfiltered whole serum and each molecular weight region included.**

Average of the 30 lymphoma patients shown here. The inset is the wavenumber region between 1800  $\text{cm}^{-1}$  and 1000  $\text{cm}^{-1}$ , which was used for all chemometrics and machine learning analyses. Spectra is offset for clearer visualisation.

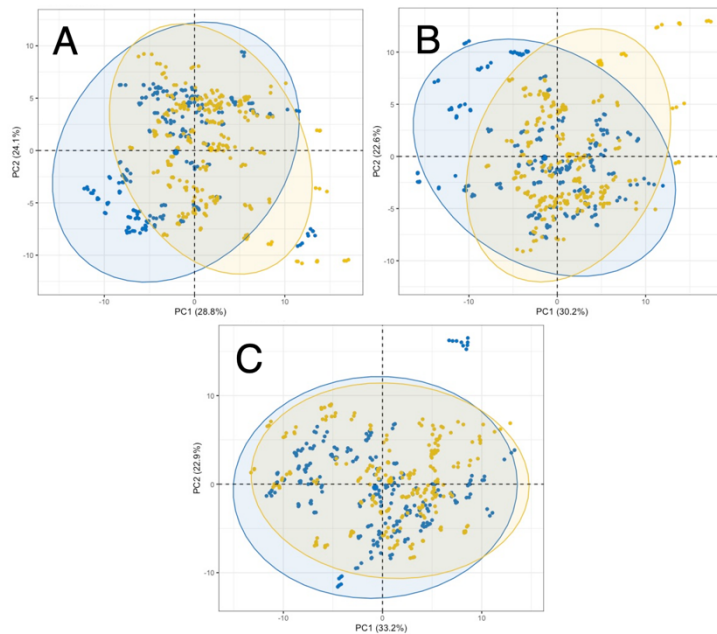


**Fig S3. Patient serum spectra including unfiltered whole serum and each molecular weight region included.**

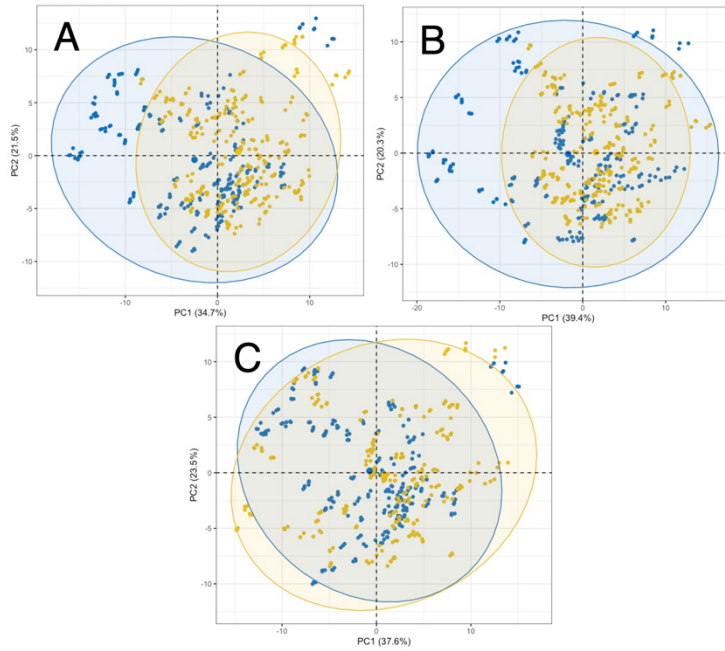
Average of the 30 non-cancer patients shown here. The inset is the wavenumber region between 1800  $\text{cm}^{-1}$  and 1000  $\text{cm}^{-1}$ , which was used for all chemometrics and machine learning analyses. Spectra is offset for clearer visualisation.



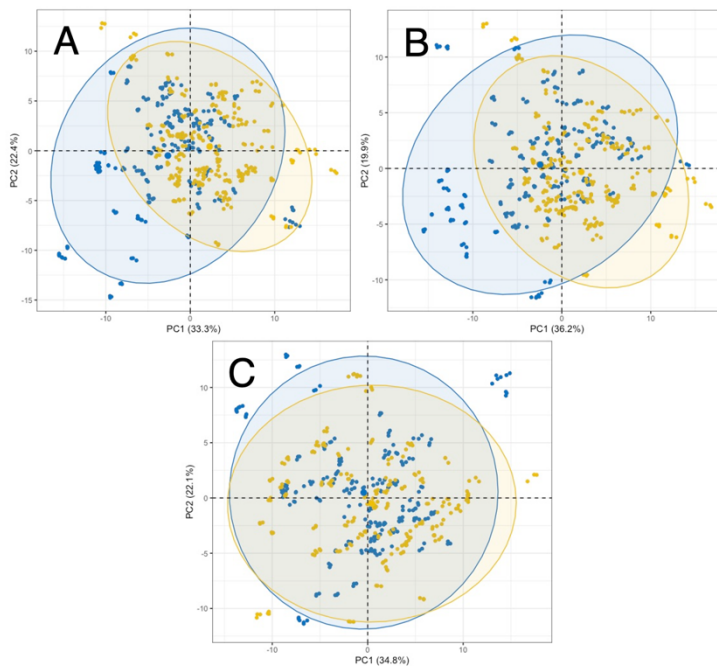
**Fig S4. PCA scores plots for the filtered serum (<50 kDa) of the first and second dimensions.** The three figures represent (A) GBM in blue and non-cancer in yellow, (B) Lymphoma in blue and non-cancer in yellow and (C) GBM in blue and lymphoma in yellow. The eclipses in each class represent a 95% confidence interval. Values in parentheses are the TEV for each PC.



**Fig S5. PCA scores plots for the filtered serum (<30 kDa) of the first and second dimensions.** The three figures represent (A) GBM in blue and non-cancer in yellow, (B) Lymphoma in blue and non-cancer in yellow and (C) GBM in blue and lymphoma in yellow. The eclipses in each class represent a 95% confidence interval. Values in parentheses are the TEV for each PC.



**Fig S6. PCA scores plots for the filtered serum (<10 kDa) of the first and second dimensions.** The three figures represent (A) GBM in blue and non-cancer in yellow, (B) Lymphoma in blue and non-cancer in yellow and (C) GBM in blue and lymphoma in yellow. The eclipses in each class represent a 95% confidence interval. Values in parentheses are the TEV for each PC.



**Fig S7. PCA scores plots for the filtered serum (<3 kDa) of the first and second dimensions.** The three figures represent (A) GBM in blue and non-cancer in yellow, (B) Lymphoma in blue and non-cancer in yellow and (C) GBM in blue and lymphoma in yellow. The eclipses in each class represent a 95% confidence interval. Values in parentheses are the TEV for each PC.

**Table S1. Sensitivity, specificity and balanced accuracies for the RF model classification of GBM *versus* non-cancer patients. Mean, standard deviation (SD) and 95% confidence intervals (CIs) are provided.**

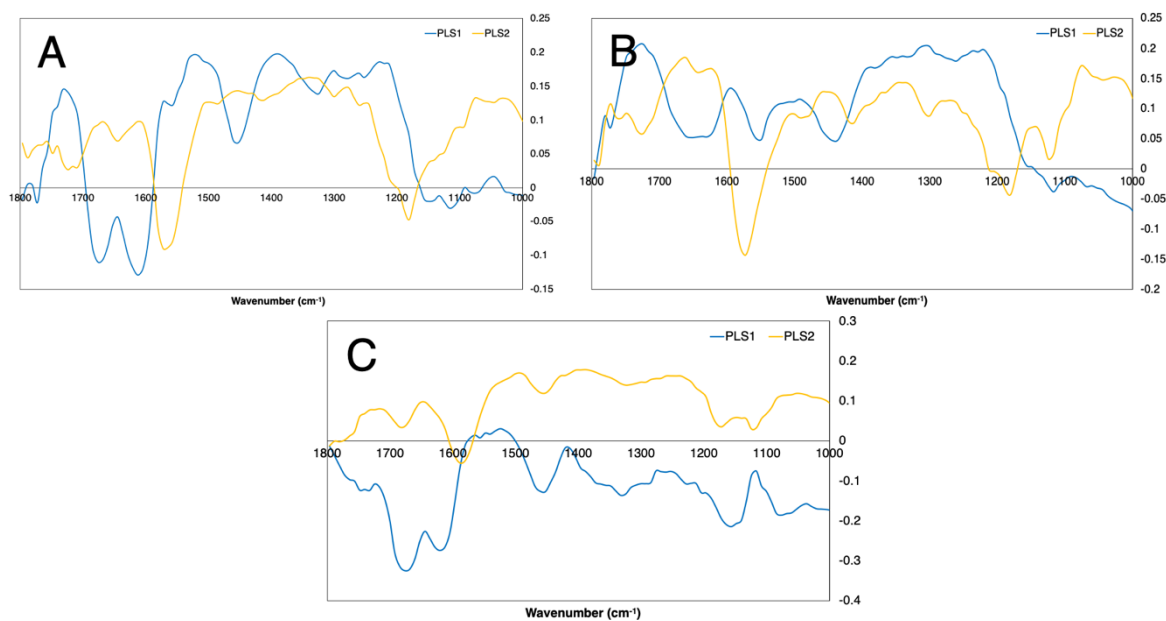
	Sensitivity (%)			Specificity (%)			Balanced accuracy (%)		
	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI
Unfiltered	88.9	9.7	$\pm 2.7$ 86.2-91.6	94.8	6.8	$\pm 1.9$ 92.9-96.7	91.8	6.0	$\pm 1.6$ 90.2-93.4
<100 kDa	85.0	14.1	$\pm 3.9$ 81.1-88.9	81.7	12.1	$\pm 3.3$ 78.4-85.0	83.4	9.5	$\pm 2.6$ 80.8-86.0
<50 kDa	73.4	16.9	$\pm 4.6$ 68.8-78.0	74.3	15.9	$\pm 4.4$ 69.9-78.7	73.8	10.6	$\pm 2.9$ 70.9-76.7
<30 kDa	76.2	17.3	$\pm 4.7$ 71.5-80.9	76.3	15.1	$\pm 4.1$ 72.2-80.4	76.2	10.0	$\pm 2.7$ 73.5-78.9
<10 kDa	69.6	17.9	$\pm 4.9$ 64.7-74.5	81.5	14.7	$\pm 4.0$ 77.5-85.5	75.5	9.3	$\pm 2.6$ 72.9-78.1
<3 kDa	78.7	18.6	$\pm 5.1$ 73.6-83.8	81.0	14.8	$\pm 4.1$ 76.9-85.1	79.9	9.2	$\pm 2.5$ 77.4-82.4

**Table S2. Sensitivity, specificity and balanced accuracies for the RF model classification of lymphoma *versus* non-cancer patients. Mean, standard deviation (SD) and 95% confidence intervals (CIs) are provided.**

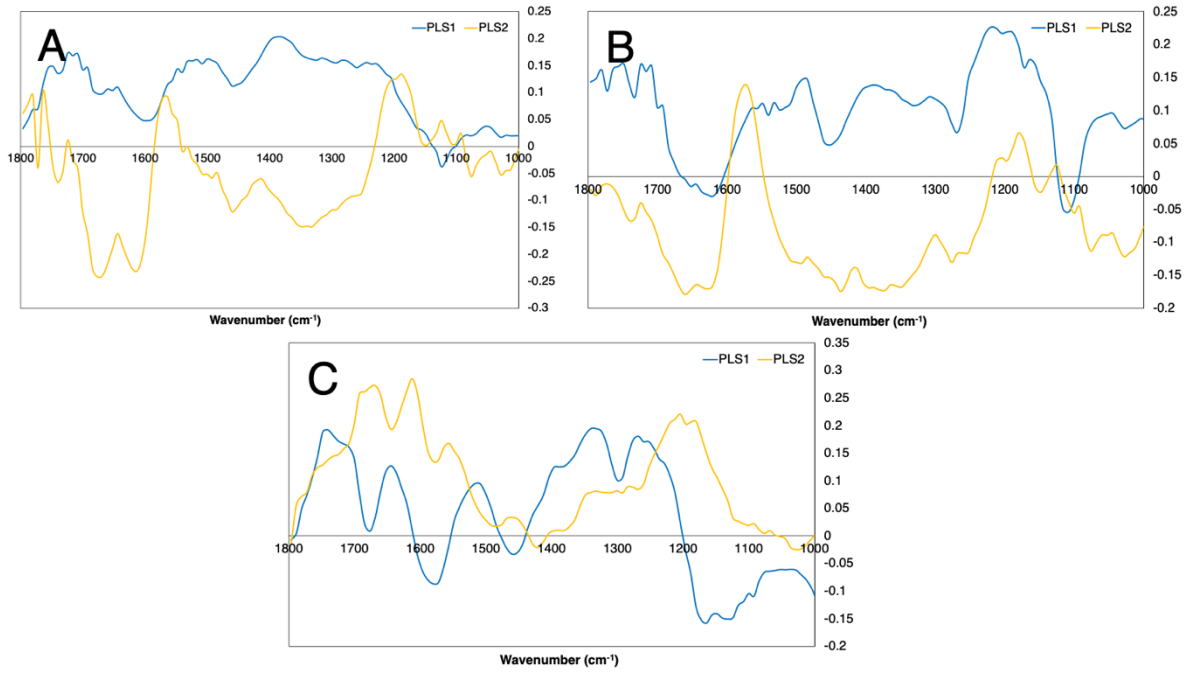
	Sensitivity (%)			Specificity (%)			Balanced accuracy (%)		
	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI
Unfiltered	83.8	13.8	$\pm 3.8$ 80.0-87.6	94.3	7.8	$\pm 2.1$ 92.2-96.4	89.1	6.9	$\pm 1.9$ 87.2-91.0
<100 kDa	59.1	20.4	$\pm 5.6$ 53.5-64.7	73.4	14.9	$\pm 4.1$ 69.3-77.5	66.3	11.6	$\pm 3.2$ 63.1-69.5
<50 kDa	54.3	17.8	$\pm 4.9$ 49.4-59.2	61.7	14.1	$\pm 3.9$ 57.8-65.6	58.0	11.1	$\pm 3.0$ 55.0-61.0
<30 kDa	59.4	18.2	$\pm 5.0$ 54.4-64.4	72.8	13.2	$\pm 3.6$ 69.2-76.4	66.1	9.7	$\pm 2.7$ 63.4-68.8
<10 kDa	58.6	16.5	$\pm 4.5$ 54.1-63.1	70.2	14.1	$\pm 3.9$ 66.3-74.1	64.4	10.1	$\pm 2.8$ 61.6-67.2
<3 kDa	67.2	17.8	$\pm 4.9$ 62.3-72.1	62.5	19.4	$\pm 5.3$ 57.2-67.8	64.8	9.2	$\pm 2.5$ 62.3-67.3

**Table S3. Sensitivity, specificity and balanced accuracies for the RF model classification of GBM *versus* Lymphoma patients. Mean, standard deviation (SD) and 95% confidence intervals (CIs) are provided.**

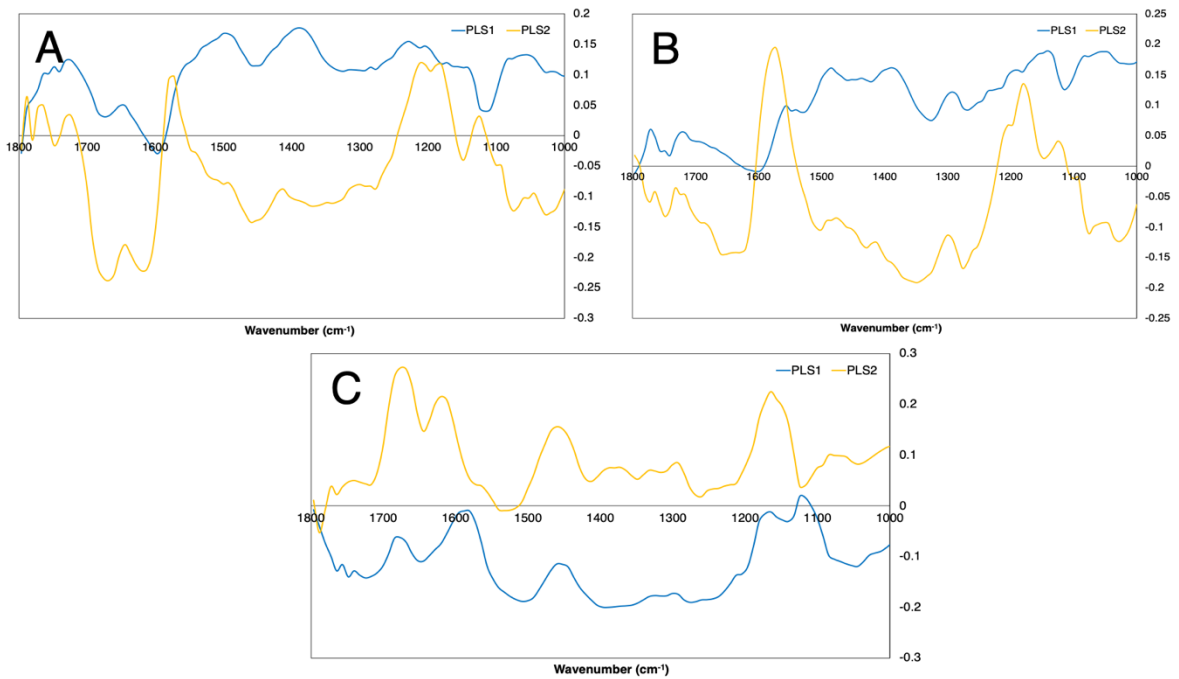
	Sensitivity (%)			Specificity (%)			Balanced accuracy (%)			
	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI	
GBM <i>versus</i> lymphoma	Unfiltered	86.3	10.4	$\pm 2.9$ 83.4-89.2	84.1	13.7	$\pm 3.8$ 80.3-87.9	85.2	8.1	$\pm 2.2$ 83.0-87.4
	<100 kDa	50.4	22.6	$\pm 6.2$ 44.2-56.6	53.5	19.3	$\pm 5.3$ 48.2-58.8	52.0	13.6	$\pm 3.7$ 48.3-55.7
	<50 kDa	49.6	20.5	$\pm 5.6$ 44.0-55.2	36.4	20.7	$\pm 5.7$ 30.7-42.1	43.0	13.4	$\pm 3.7$ 39.3-46.7
	<30 kDa	57.8	20.8	$\pm 5.7$ 52.1-63.5	45.7	20.8	$\pm 5.7$ 40.0-51.4	51.8	12.1	$\pm 3.3$ 48.5-55.1
	<10 kDa	38.5	14.3	$\pm 3.9$ 34.6-42.4	47.9	15.1	$\pm 4.1$ 43.8-52.0	43.2	9.4	$\pm 2.6$ 40.6-45.8
	<3 kDa	50.0	18.0	$\pm 4.9$ 45.1-54.9	39.2	19.6	$\pm 5.4$ 33.8-44.6	44.6	11.5	$\pm 3.2$ 41.4-47.8



**Fig S8. PLS loadings plot for the 1st and 2nd LVs for the filtered serum (<50 kDa).**  
(A) GBM *versus* non-cancer, (B) Lymphoma *versus* non-cancer and (C) GBM *versus* lymphoma.

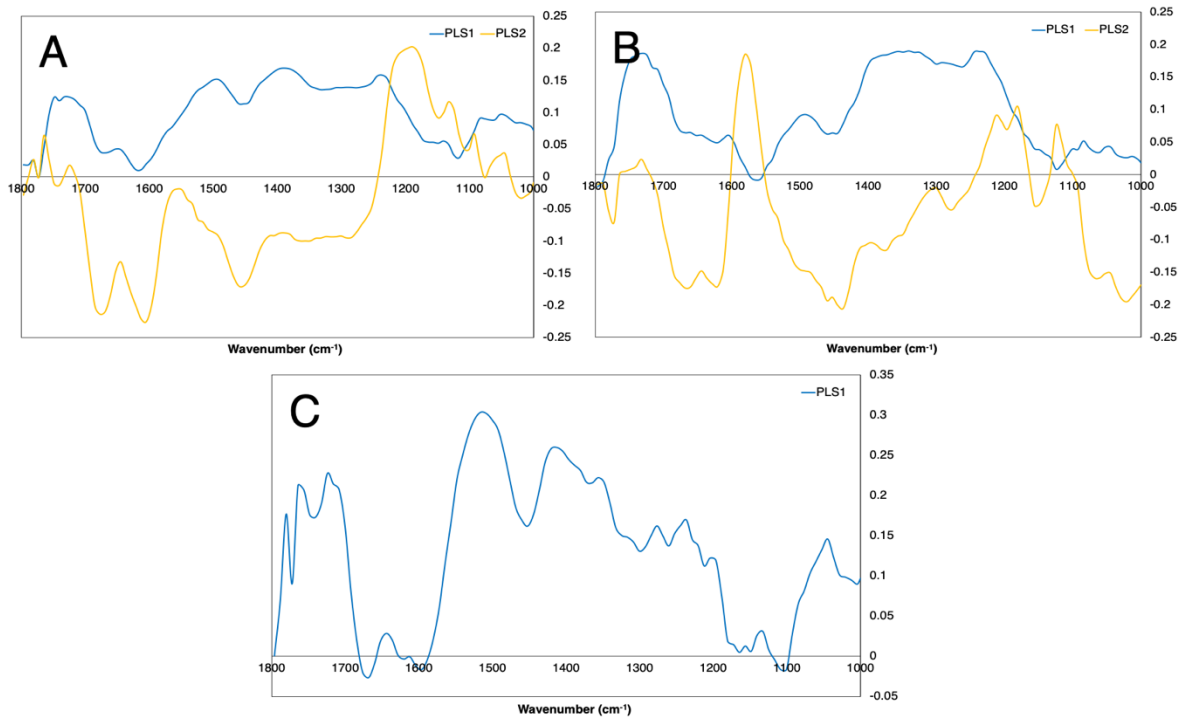


**Fig S9. PLS loadings plot for the 1st and 2nd LVs for the filtered serum (<30 kDa).**  
 (A) GBM *versus* non-cancer, (B) Lymphoma *versus* non-cancer and (C) GBM *versus* lymphoma.



**Fig S10. PLS loadings plot for the 1st and 2nd LVs for the filtered serum (<10 kDa).**  
 (A) GBM *versus* non-cancer, (B) Lymphoma *versus* non-cancer and (C) GBM *versus* lymphoma.





**Fig S11. PLS loadings plot for the 1st and 2nd LVs for the filtered serum (<3 kDa).**  
 (A) GBM *versus* non-cancer, (B) Lymphoma *versus* non-cancer and (C) GBM *versus* lymphoma.