

## 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                                                                                                                                 |

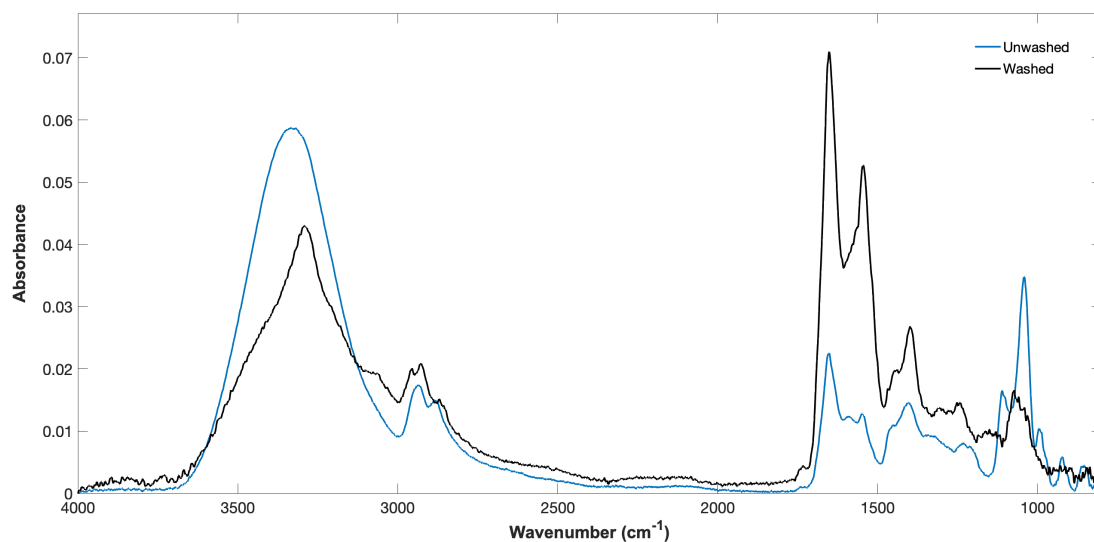
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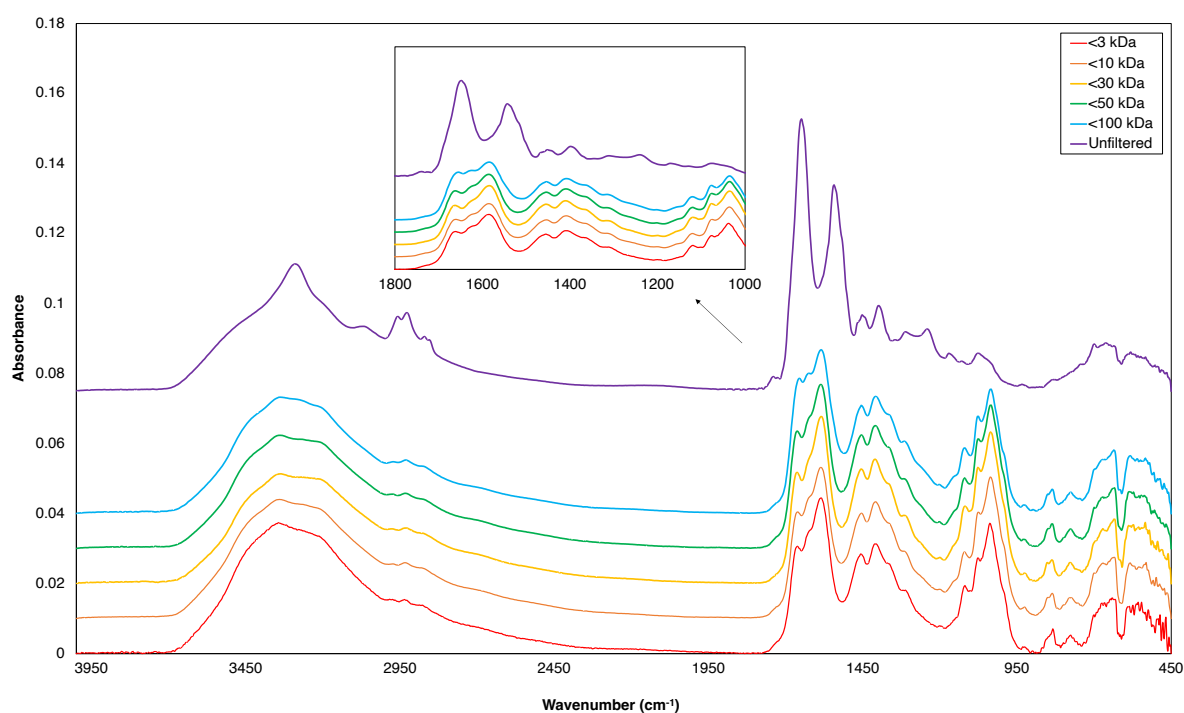
# Investigating centrifugal filtration of serum-based FTIR spectroscopy for the stratification of brain tumours

## SUPPORTING INFORMATION



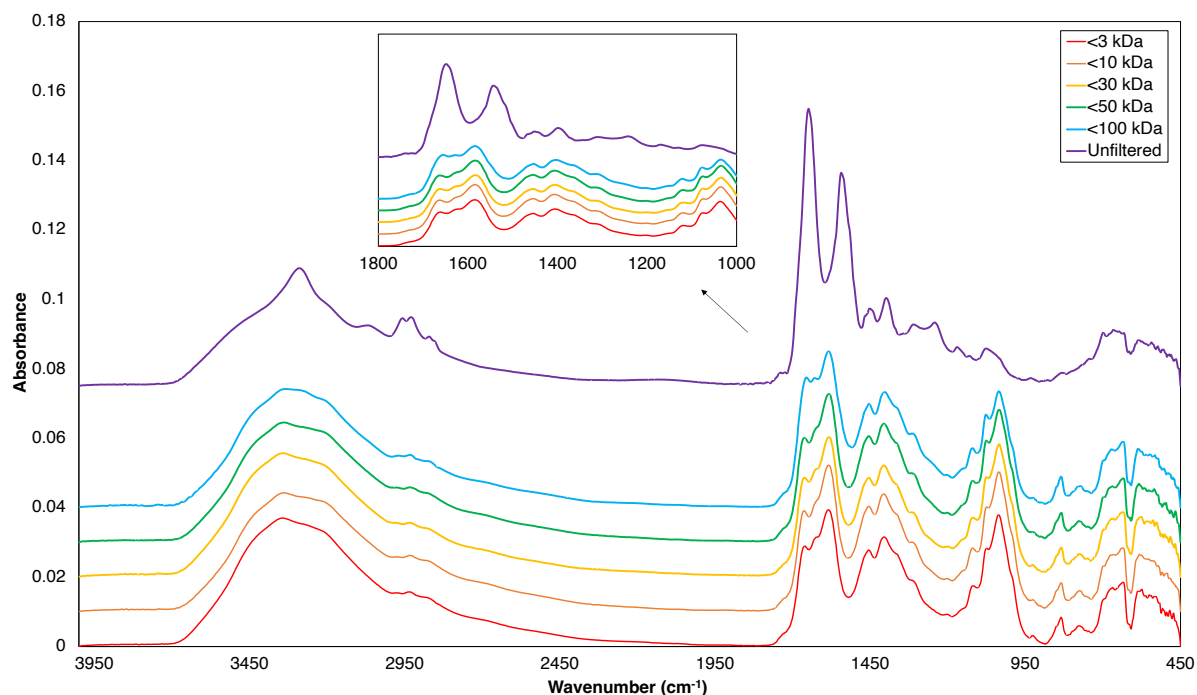
**Fig S1. Example spectra of a non-cancer patient.**

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.



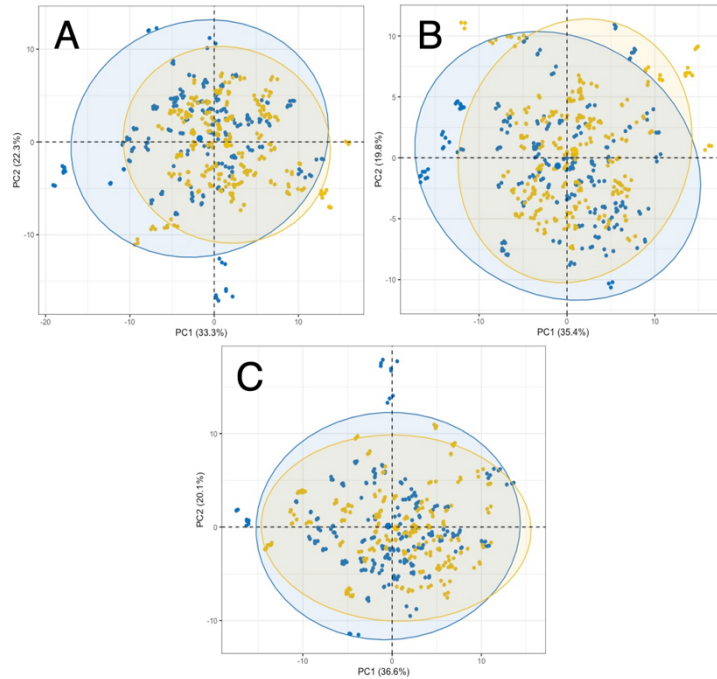
**Fig S2. Patient serum spectra including unfiltered whole serum and each molecular weight region included.**

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.

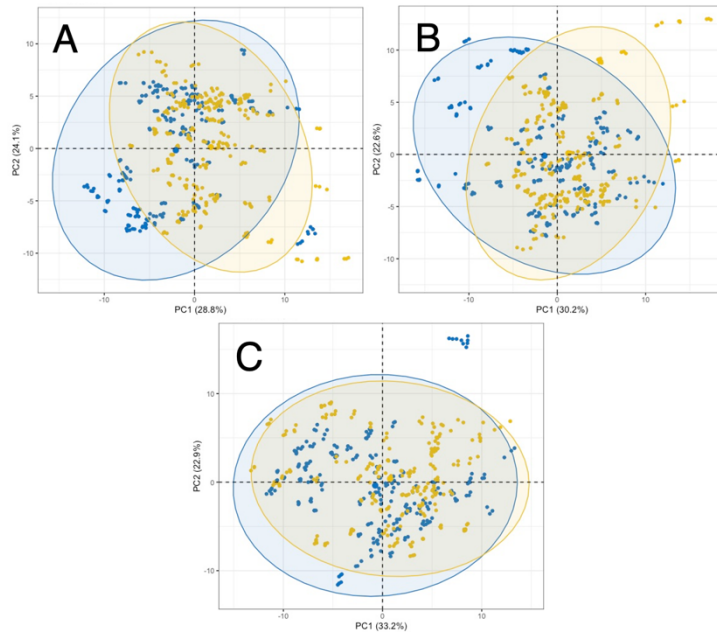


**Fig S3. Patient serum spectra including unfiltered whole serum and each molecular weight region included.**

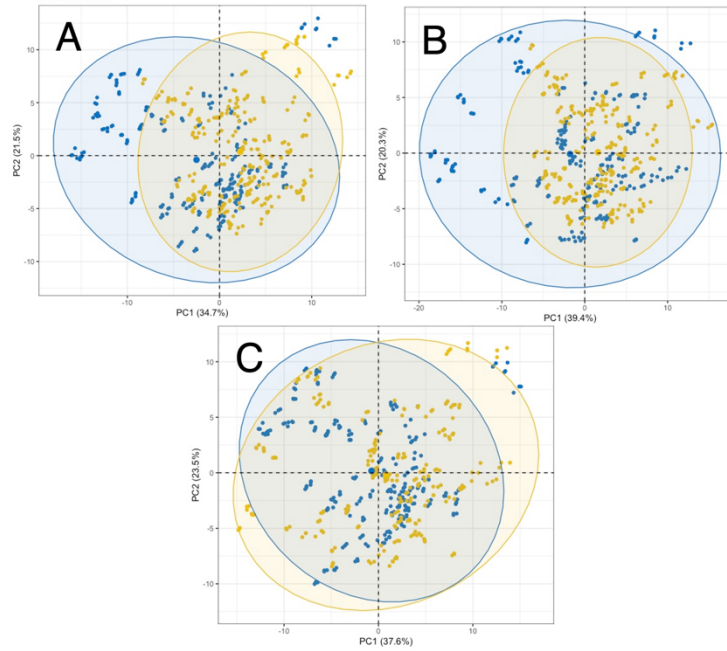
Average of the 30 non-cancer patients shown here. The inset is the wavenumber region between 1800  $\text{cm}^{-1}$  and 1000  $\text{cm}^{-1}$ , which was used for all chemometrics and machine learning analyses. Spectra is offset for clearer visualisation.



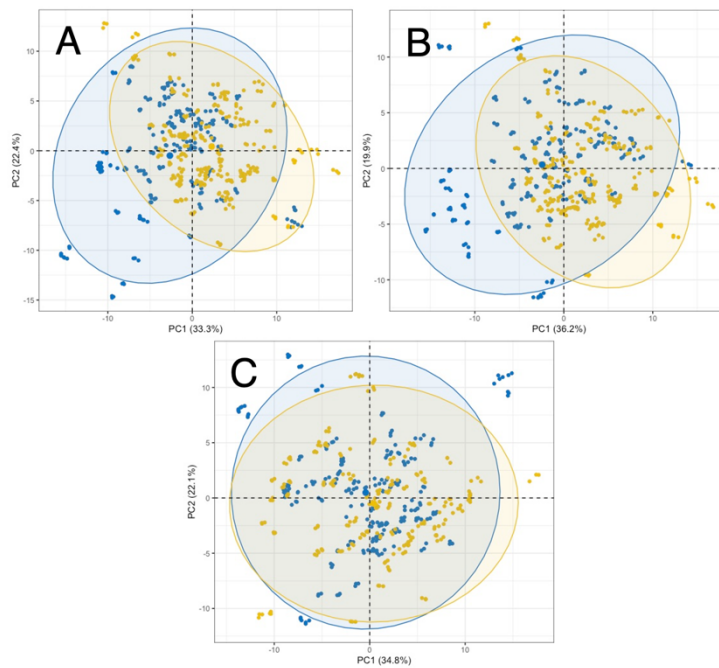
**Fig S4. PCA scores plots for the filtered serum (<50 kDa) of the first and second dimensions.** The three figures represent (A) GBM in blue and non-cancer in yellow, (B) Lymphoma in blue and non-cancer in yellow and (C) GBM in blue and lymphoma in yellow. The eclipses in each class represent a 95%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

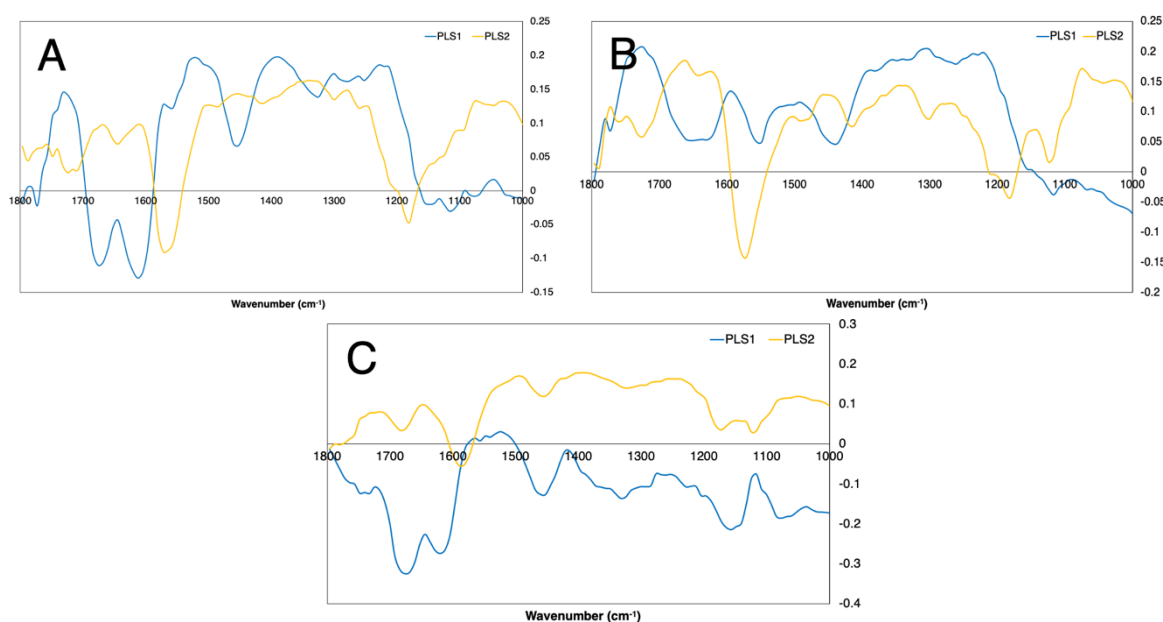
|                            |            | Sensitivity (%) |      |                        | Specificity (%) |      |                        | Balanced accuracy (%) |      |                        |
|----------------------------|------------|-----------------|------|------------------------|-----------------|------|------------------------|---------------------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

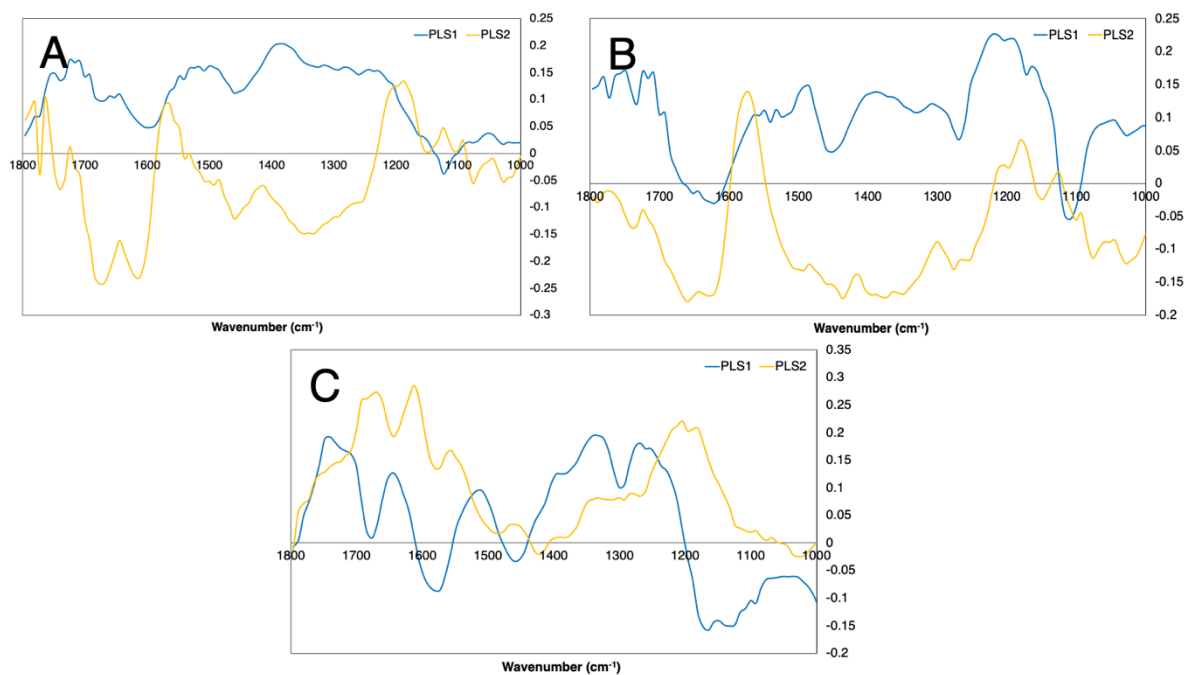
|                                 |            | Sensitivity (%) |      |                        | Specificity (%) |      |                        | Balanced accuracy (%) |      |                        |
|---------------------------------|------------|-----------------|------|------------------------|-----------------|------|------------------------|-----------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

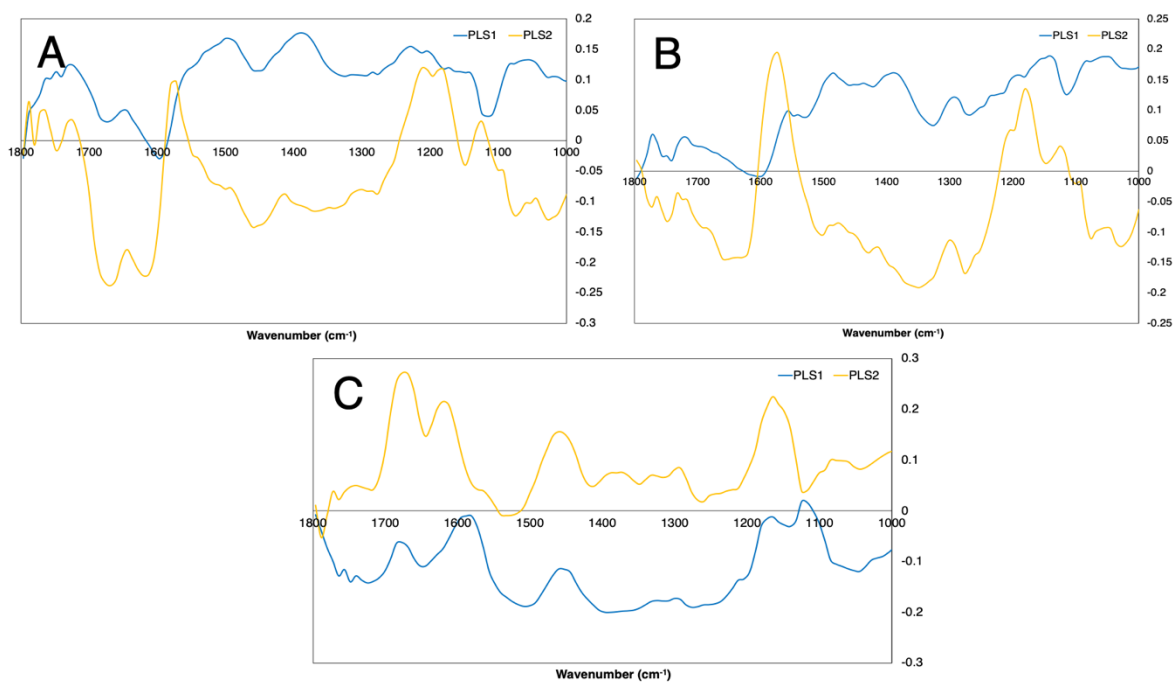
|                           |            | Sensitivity (%) |      |                        | Specificity (%) |      |                        | Balanced accuracy (%) |      |                        |
|---------------------------|------------|-----------------|------|------------------------|-----------------|------|------------------------|-----------------------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



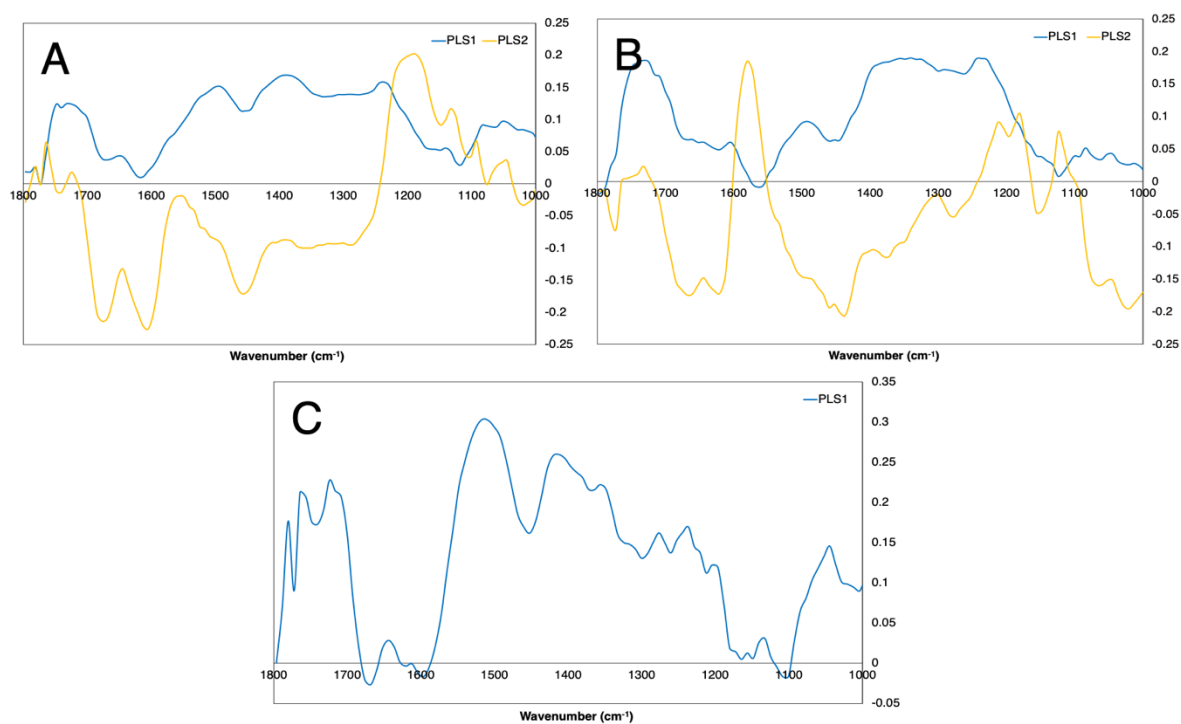
**Fig S8. PLS loadings plot for the 1st and 2nd LVs for the filtered serum (<50 kDa).**  
(A) GBM *versus* non-cancer, (B) Lymphoma *versus* non-cancer and (C) GBM *versus* lymphoma.



**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.