RCPAQAP Molecular Genetics

Quality use of Pathology Program

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The development of a mass spectrometry technical quality assurance program for detecting human disease-associated proteins.

Final Report

A project funded under the Australian Government's Quality Use of Pathology Program

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Executive Summary

Genetic disease diagnostic testing is primarily focused on identifying gene DNA variations that are associated with a pathological disorder. However, accumulating evidence from whole genome (entire human DNA) and whole exome (gene coding DNA regions only) sequencing indicates that there are many 1000s of DNA variations found in healthy individuals and in individuals with a pathological disorder. Since many of the same DNA variants are found in healthy individuals and in a genetic pathology, the diagnostic evaluation of a small number of DNA gene variants alone may not necessarily indicate or confirm an underlying disease process. Such findings may therefore be difficult to fully clinically interpret. A more efficient process for aiding the clinician for interpretation of disease is to link DNA gene variation with both protein expression and protein variant identification. Such diagnostic data would provide evidence that a DNA gene variant is being expressed at the protein level and may therefore be functionally relevant to the disease process. This represents a novel approach for genetic disease diagnostics. Although protein testing is available for bacterial and viral infections, proteomic testing for genetic disease remains in its infancy. Importantly, laboratories using the technique of mass spectrometry have identified key proteins associated with various human genetic diseases. These data therefore provide evidence that proteomic diagnostic testing for genetic disorders are becoming a viable reality.

Purpose

The purpose of this study was to therefore assess a small number of facilities using the technique of protein mass spectrometry for the detection of human specific proteins. The underlying principle for this pilot study was to identify in the short-term, key areas of problems associated with human protein testing and to determine the level of consistency of laboratory reporting for the proteins tested for. The data from this pilot can then be used to formulate a medium- to long-term strategy for the development of an external quality assurance (EQA) program for this state-of-the art application for human genetic disease diagnostics. Although funding from the Commonwealth for such an early phase pilot study may not be fully representative a standard business model for a QUPP award, the preliminary data produced are nonetheless pivotal for providing key information for the long-term development of a fully operation quality assurance program. Importantly, before any human protein genetic disease diagnostics can be offered and incorporated into the Medicare Benefits Schedule (MBS), an EQA proficiency testing program first needs to be available. Commonwealth funding for new areas of diagnostic developments that are of benefit for long-term patient care are therefore essential as this allows the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) to devise and develop novel EQAs alongside current pathological discoveries so that clinical and patient needs can be met and continually addressed.

For this RCPAQAP initiative pilot study, a total of 70 peptides (containing natural wild-type peptides and peptides isotopically labelled with $^{13}C/^{15}N$ at the C-terminal arginine or lysine peptide) were distributed to five mass spectrometry testing laboratories. 35 peptides (samples A, B, C) were uncorrelated (i.e., were a random generation of wild-type to labelled peptides), and 35 peptides (samples D, E, F) were correlated (i.e., known ratios of wild type to labelled peptide). Four laboratories (80%) successfully submitted data and one laboratory withdrew from the study due to unforeseen laboratory restructuring. The four laboratories that submitted data also encountered problems with the reference testing samples. For example, laboratories reported on high backpressure build-up on peptide filtering columns, and some peptides generally being of poor quality for adequate detection. These issues caused significant delays for result generation and for returning data to the RCPAQAP. However, such laboratory feedback is key information for the RCPAQAP since it allows for modification of future protein reference standard design.

For proficiency testing of the submitted peptide data, the RCPAQAP used a modified similarity and dissimilarity data mining statistical approach. This allowed for laboratory data to be directly compared against the reference standard data for (i) similarity testing (i.e., full peptide identification in comparison to the reference data) and (ii) dissimilarity testing (i.e., comparison to the reference data of the wild-type to labelled peptide ratios). Percentage values were then derived for final comparative analysis against the expected EQA reference data. In total, none of the four laboratories fully identified all peptides in all samples. For peptide similarity testing, laboratory peptide identification ranged from 66% to 97% similar in comparison to the reference data. For peptide ratio dissimilarity testing, ratio values ranged from 3% to 96% dissimilar in comparison to the reference data. In addition, the correlated samples (D, E, F) were more readily identified by laboratories than the uncorrelated samples (A, B, C) and may suggest an issue with the EQA reference samples, or with each laboratory's mass spectrometer calibration setup. The advantage of using the similarity dissimilarity statistical approach for genetic protein diagnostic analysis is that it allows us to identify the performance of each laboratory for their ability to (i) detect and identify unknown peptides, and (ii) to determine each laboratories ability to characterise reference peptide ratio values. This strategy is key for providing constructive feedback to each laboratory's performance so that areas of concern can be identified and improved.

The current Commonwealth funded project has allowed the RCPAQAP to identify key areas for improvement for the development an EQA for human protein diagnostics. In the short-term, the data generated from this study highlighted two primary areas for improvement. Firstly, the EQA reference material needs to be of better quality since each laboratory reported similar encountered problems. Secondly, there appears to be calibration issues relating to the mass spectrometry equipment used

given that some laboratories were less proficient at detecting the unknown peptides and ratio values. Nonetheless, the data produced indicates that protein diagnostics using human peptides is certainly possible. In the mid-term, a second study is now required to encompass a larger cohort of laboratories. Such a second study allows the RCPAQAP to address the findings from the current study and improve on the development of a protein specific EQA. In the long-term, an EQA for protein mass spectrometry will be key for diagnosing the complex diseases of cancer (i.e., breast, lung, prostate gastrointestinal), neurological disorders (i.e., Alzheimer, Parkinson, Huntington, epilepsy), and age-related disease (i.e., cardiovascular disease). Thus, proteomic results in combination with identified genetic alterations will allow clinicians to make better informed decisions with respect to pharmaceutical intervention and patient management.

Aim of the Study

(*What is the aim/purpose of the project?*)

The primary aim of this study was to assess facilities using the technique of protein mass spectrometry for the detection of human peptides. The reason being that key malfunctioning proteins have been detected in various human diseases and proteomic diagnostic testing is therefore becoming a viable reality. However, before any proteomic diagnostics can be offered, an EQA proficiency testing program needs to be available. The RCPAQAP therefore devised and performed an EQA trial study to determine the ability of mass spectrometry testing facilities to correctly identify a range of unknown reference testing protein standards.

Background

(What is the overview of the project and its importance for disease diagnostics?)

Identifying abnormal proteins in response to genetic DNA variation, microbial infection, or as a consequence of another underlying disease pathology is key to understanding the human disease process. As such, key organisations are now aiming to identify human gene expressed proteins. In particular, the Human Proteome Organization (HUPO) are currently performing a study designed to map the entire human proteome using the technique of mass spectrometry together with new and emerging technologies. Completion of the HUPO project is expected to enhance our understanding of human biology at the cellular level and to provide a foundation for future developments of diagnostic, prognostic, therapeutic, and preventive medical applications. HUPO have currently identified 93% of the human proteome with 19,823 human proteins being categorised (https://www.hupo.org/).

In addition to the HUPO study, protein biomarkers are also being increasingly recognised in different human diseases. For example, rapid improvements in mass spectrometry technology has enabled the identification of key proteins associated with kidney disease, lung disease, and cancer (Picken 2015; Pontillo and Mischak 2017; Callejón-Leblic et al., 2016; Jones et al., 2016; Ohlmeier et al., 2016). Disease-specific biomarker proteins have also been found to be circulating in blood and to be excreted in urine (Bansal et al., 2016; Chen and Kim, 2016; Lu et al., 2016). These tissues are termed liquid biopsy tissue and the key advantage for proteomic diagnostic testing of these is that the invasive procedures of pathology tissue excision and tissue biopsy do not need to be performed which is more efficient and much less stressful for the patient. Importantly, a protein liquid biopsy test has now been developed for the detection of circulating blood proteins that are associated with cancer (https://www.biotechsupportgroup.com/Stroma-Liquid-Biopsy-s/287.htm). Tests such as these will further enhance a laboratory's ability to detect cancer at a much earlier stage. Given the plethora of proteins being identified in disease, there is now a need and demand for the development of proteomic diagnostic testing. It is therefore essential that an EQA program be devised so that proficiency testing can be offered to laboratories for near future clinical proteomic diagnostics.

This project was therefore designed to quality assess the technical ability of laboratories to accurately detect and report on protein targets using the high-throughput sensitive technique of mass spectrometry. The development of a proteomic EQA will allow laboratories to enrol for proficiency testing in this new key area of clinical diagnostics.

Addressing essential needs

(*What need/s will this project address?*)

Recent publications (including data presented at international and national conferences) reveal that there is a high need for quality metrics to be designed for monitoring of mass spectrometry proteomic analysis (Hoofnagle et al., 2016; Sanchez-Niño et al., 2017). Furthermore, with HUPO nearing completion of the human proteome, clinical proteomic diagnostic testing is becoming more likely (Sanchez-Niño et al., 2017). Importantly, combining genetic and proteomic analyses in the near future will enable greater clinical diagnostic power and disease prediction, and may also allow for early detection of underlying molecular disease processes (Cohen et al., 2017, 2018). There is therefore an essential requirement to meet technological developments and diagnostic improvements with an external quality assurance program so consistency of diagnoses can be monitored. This is of key concern especially if laboratories inadvertently miscall protein expression or post translational modifications and provide false negative or false positive reports to the referring clinician. In addition, there is an unmet need in post market surveillance for monitoring disease-specific proteins

associated with treatment resistance in response to pharmaceutical intervention. This critical information will aid in the key decision-making process for patient treatment.

Benefits

(*What benefit will the project be to consumers of pathology services?*)

Proteomic testing in disease pathology is growing and represents an essential strategy to determine key underlying molecular mechanisms involved in the disease process. The identification of protein biomarkers in blood serum and urine is causing a shift in thinking away from the traditional practise of tissue excision/biopsy to now focus on circulating or excreted proteins for diagnostic characterisation. This non-invasive nature of testing makes it a very attractive technique over the invasiveness of tissue excision/biopsy surgery and of the risks associated with this. A quality assurance program allows laboratories to directly compare data so that problems can be rectified, and levels of consistency can be maintained.

Proteomics tests can generate complex data and understanding their clinical significance is key as this will reflect in the clinical management of the patient. The ability to monitor the levels of circulating or excreted proteins in response to ongoing pharmaceutical intervention will help address the clinical significance between pharmaceutical treatment and patient response. This is particularly important in the monitoring of proteins that are associated with pharmaceutical intervention resistance (i.e., the epidermal growth factor receptor (EGFR) protein variant (p.Thr790Met) in non-small cell lung cancer) or in the monitoring of a patient's response to compounds designed to overcome such tumour resistance (Kobayashi et al 2005; Ku et al., 2016). The need for quality assurance is therefore critical since the accuracy of these diagnostic tests will greatly aid the clinician for appropriate patient management. Furthermore, early patient diagnosis may improve clinical treatment and significantly reduce associated healthcare costs.

Quality monitoring disease-associated proteins will ultimately allow clinicians to make better informed decisions with respect to pharmaceutical intervention and patient management. The RCPAQAP also provide valuable educational components, which are produced in collaboration with key stakeholders (including pathologists) and members of the RCPAQAP genetics Advisory Committee. These are of benefit to pathology communities comprising scientists, clinical geneticists, genetics pathologists and oncologists.

Reference samples

(*What were the samples used in the project*)

The reference testing peptide samples used for this project were designed and developed by MRM Proteomics (https://mrmproteomics.com/). Two sets of reference peptide mixes were produced. Each reference mix contained 35 standard synthetic peptides in two different isotopic forms: natural abundance (light peptide) and isotopically labelled with $^{13}C/^{15}N$ at the C-terminal arginine or lysine (Stable Isotope-labelled Standard peptide or SIS peptide). Each set of reference peptide mixes include three different samples, where the concentration of light peptides varies, but the concentration of SIS peptides remains constant. The concentrations of each peptide relative to other peptides vary. In the first set of peptides the light/SIS peptide ratios are uncorrelated, meaning that each peptide ratio varies with respect to other peptides in the sample and also between reference samples in the same set. The second set of reference samples are correlated, meaning that the light/SIS peptide ratios are similar between each peptide in the same reference sample and also vary similarly between reference samples. Table 1 below summarizes the reference samples used in the study and distributed to each laboratory. The proteins and peptide sequences are provided in the Appendix.

Table 1. Reference testing samples used for mass spectrometry proficiency testing.

Reference Set	Reference sample	Aliquots provided (tubes)	Aliquot volumes (µl)
Uncorrelated	A	40	50
Uncorrelated	B	40	50
Uncorrelated	C	40	50
Correlated	D	40	50
Correlated	E	40	50
Correlated	F	40	50

Methods

(*Technical background of the study*)

1. Laboratories

A total of five mass spectrometry facilities agreed to participate in the RCPAQAP trial proteomic proficiency testing program. These facilities were based at Western Australia (two laboratories), New South Wales (two laboratories), and South Australia (one laboratory).

2. Peptide mix preparation

Stocks of the various peptide standards (both light and SIS) were pooled together to produce the two sets of peptide reference samples. The mixes were prepared, lyophilized and rehydrated in digested bovine serum albumin (BSA) in 0.1% formic acid before aliquoting. The presence of peptides from digested BSA helps reduce the potential variability due to adsorption of peptide to labware and provides a more representative background matrix for the analysis of the proteomics reference sample. Briefly, the two sets of reference samples were prepared with the following guidelines:

Uncorrelated Reference Samples (A, B, C)

i. Each sample contained 35 peptides in the SIS & light form.
ii. The concentration of each SIS peptide was fixed across the three A, B, C reference samples.
iii. The concentration of each light peptide varied across the three A, B, C reference samples.
iv. The ratios of each SIS peptide to its corresponding light peptide for the three reference samples was quasi-random. There was an approximate 25-fold range for the concentration of each peptide across the three samples.
v. The concentrations for each peptide in a given reference sample are uncorrelated to those in the other reference samples.

Correlated Reference Samples (D, E, F)

i. Each sample contained 35 peptides in the SIS & light forms.
ii. The concentration of each SIS peptide was fixed across the three D, E, F reference samples.
iii. The concentration of each light peptide varied across the three D, E, F reference samples.
iv. The ratios of each SIS peptide to its corresponding light peptide for the three reference samples was approximately 1:0.2, 1:1, 1:5; giving a 25-fold range for the concentration of each peptide across the 3 samples.

3. Reference testing of peptide samples

Five replicate injections of 10 µL of each reference sample were analysed by liquid chromatography–mass spectrometry (LC-MS). Samples were separated with a Zorbax Eclipse Plus RP-UHPLC column (2.1 x 150 mm, 1.8 µm particle diameter; Agilent) with a 1290 Infinity system (Agilent). Peptide separations were achieved at 0.4 mL/min over a 60 min run, via a multi-step LC gradient (2-80% mobile phase B; mobile phase compositions: A was 0.1% FA in water while B was 0.1% FA in acetonitrile). The column was maintained at 50°C. A post-gradient column re-equilibration of 4 min was used after each sample analysis.

The LC system was interfaced to a triple quadrupole mass spectrometer (Agilent 6495B) via a standard-flow AJS ESI source, operated in the positive ion mode. The general MRM acquisition parameters employed were as follows: 3.5 kV capillary voltage, 300 V nozzle voltage, 11 L/min sheath gas flow at a temperature of 250 °C, 15 L/min drying gas flow at a temperature of 150 °C, 30 psi nebulizer gas pressure, 380 V fragmentor voltage, 5 V cell accelerator potential, and unit mass resolution in the first and third quadrupole mass analysers. The high energy dynode (HED) multiplier was set to -20 kV for improved ion detection efficiency and signal-to-noise ratios. Specific LC-MS acquisition parameters were employed for optimal peptide ionization/fragmentation and scheduled MRM. Note that the peptide optimizations were empirically optimized previously by direct infusion of the purified SIS peptides. In the quantitative analysis, the targets were monitored over 1.5 min detection windows.

The MRM data was visualized and examined with Skyline Daily Quantitative Analysis software (version 3.7.1.11571, University of Washington). This involved peak inspection to ensure accurate selection, integration, and uniformity (in terms of peak shape and retention time) of the SIS and light peptides. The average light/SIS peptide ratios were calculated and the precision of the five measurements were tabulated (expressed as %CV) using the sum of the top three MRM transitions.

4. RCPAQAP Proficiency test analysis

To determine the proficiency of each laboratory for detecting unknown protein peptides, each laboratory's data were directly compared against the MRM Proteomic reference standard data to firstly confirm that the identity of each peptide was correct, and secondly, to identify that the ratios of the two sets of 35 peptides matched that of reference data. For analysis, an RCPAQAP proficiency test scoring system was devised using a modified similarity dissimilarity statistical test (Clarke, 1993). A percentage comparison to the reference standard data could therefore be derived for similarity testing (i.e., peptide identification) and dissimilarity testing (i.e., peptide ratio values). In this way, the proficiency of each laboratory for identifying unknown peptides and peptide ratio values

could be fully determined. These data are key for diagnostic purposes given that novel peptides may be associated with different diseases.

Coefficients of similarity and dissimilarity calculation (Example):

Protein	Reference Ratio	Laboratory Ratio	Difference in Ratios	Sum of Ratios
Protein 1	0.098	0.111	0.013	0.209
Protein 2	0.063	0.065	0.002	0.128
Protein 3	0.042	0.049	0.007	0.091
Proteins				
Detected	3	3		
Sum	0.022	0.428		

Similarity (protein identification) 3/3 = 1 (100%)

Dissimilarity (protein ratio identification) 0.022/0.428 = 0.05 (5%)

In the above example, the laboratory identified the same proteins as contained in the reference (i.e. were 100% similar) and their ratio values to the reference ratio values were only 5% dissimilar.

Results

(*Results produced from the study*)

1. Laboratories

Of the five laboratories agreeing to participate in this trial proficiency test, 80% (4/5) submitted data and one laboratory withdrew from the study.

2. RCPAQAP Proficiency testing analysis

(i) Identification of peptide sequence for uncorrelated standards (Samples A, B, C)

Each laboratory submitted their peptide sequence data. For the uncorrelated proteins, Laboratory 1 could detect all reference sequences except for the Alpha-1-antichymotrypsin peptide (Table 2). Laboratory 2 could not perform a test for the uncorrelated samples and were not assessed, Laboratory 3 detected 23 peptides and Laboratory 4 detected 30 peptide sequences (Table 2).

(ii) Identification of peptide sequence for correlated standards (Samples D, E, F)

For the correlated proteins, Laboratory 1 did not perform testing of Sample F but could detect all reference sequences in Samples D and E except for the Alpha-1-antichymotrypsin peptide (Table 3). Laboratory 2 detected 33 peptides, Laboratory 3 detected 30 peptides and Laboratory 4 detected 30 peptide sequences (Table 3).

(iii) RCPAQAP proficiency test (PT) scoring of all protein standards (Samples A - F)

Laboratory similarity dissimilarity raw data and PT scores for the detection of each protein are listed in Tables 4 - 9. The percentage of similarity (protein identification) and dissimilarity (protein ratios) for each sample tested are presented in Table 10.

Table 2. Confirmation of laboratory detection for the reference testing of uncorrelated peptides.

Uncorrelated Proteins	**Peptide Sequence**	**Protein Sequence**	**Protein Sequence**	**Protein Sequence**	**Protein Sequence**
		Lab 1	**Lab 2**	**Lab 3**	**Lab 4**
Adiponectin	IFYNQQNHYDGSTGK	YES	NA	YES	YES
Afamin	DADPDTFFAK	YES	NA	NO	YES
Alpha-1-acid glycoprotein 1	NWGLSVYADKPETTK	YES	NA	YES	YES
Alpha-1-antichymotrypsin	EIGELYLPK	NO	NA	NO	YES
Alpha-2-antiplasmin	LGNQEPGGQTALK	YES	NA	YES	YES
Alpha-2-macroglobulin	AIGYLNTGYQR	YES	NA	YES	YES
Apolipoprotein A-I	ATEHLSTLSEK	YES	NA	YES	YES
Apolipoprotein A-IV	LGEVNTYAGDLQK	YES	NA	YES	YES
Apolipoprotein B-100	FPEVDVLTK	YES	NA	YES	YES
Apolipoprotein E	LGPLVEQGR	YES	NA	YES	YES
Attractin	SVNNVVVR	YES	NA	NO	YES
Beta-2-glycoprotein 1	ATVVYQGER	YES	NA	YES	YES
Biotinidase	SHLIIAQVAK	YES	NA	NO	NO
Carbonic anhydrase 1	VLDALQAIK	YES	NA	NO	YES
CD5 antigen-like	LVGGLHR	YES	NA	NO	YES
Cholinesterase	YLTLNTESTR	YES	NA	YES	NO
Clusterin	ELDESLQVAER	YES	NA	YES	YES
Coagulation factor XII	EQPPSLTR	YES	NA	NO	NO
Complement C1r subcomponent	GLTLHLK	YES	NA	NO	YES
Complement C3	TGLQEVEVK	YES	NA	YES	YES
Complement component C9	LSPIYNLVPVK	YES	NA	YES	YES
Complement factor B	EELLPAQDIK	YES	NA	NO	YES
Fibulin-1	TGYYFDGISR	YES	NA	NO	YES
Hemoglobin subunit alpha	VGAHAGEYGAEALER	YES	NA	NO	YES
Hemopexin	NFPSPVDAAFR	YES	NA	YES	YES
Heparin cofactor 2	TLEAQLTPR	YES	NA	YES	YES
Hyaluronan-binding protein 2	VVLGDQDLK	YES	NA	YES	YES
Inter-alpha-trypsin inhibitor heavy chain H2	SLAPTAAAK	YES	NA	YES	YES
Kininogen-1	TVGSDTFYSFK	YES	NA	YES	YES
Pigment epithelium-derived factor	LQSLFDSPDFSK	YES	NA	YES	YES
Plasma protease C1 inhibitor	FQPTLLTLPR	YES	NA	YES	NO
Plasminogen	LFLEPTR	YES	NA	NO	YES
Prothrombin	ELLESYIDGR	YES	NA	YES	NO
Serotransferrin	DGAGDVAFVK	YES	NA	YES	YES
Vitronectin	FEDGVLDPDYPR	YES	NA	YES	YES

*NA (not assessed). Detection not applicable to Lab 2 as testing of the uncorrelated samples could not be performed.

Table 3. Confirmation of laboratory detection for the reference testing of correlated peptides

Uncorrelated Proteins	Peptide Sequence	Protein Sequence	Protein Sequence	Protein Sequence	Protein Sequence
		Lab 1	Lab 2	Lab 3	Lab 4
Adiponectin	IFYNQQNHYDGSTGK	YES	YES	YES	YES
Afamin	DADPDTFFAK	YES	YES	YES	YES
Alpha-1-acid glycoprotein 1	NWGLSVYADKPETTK	YES	YES	YES	YES
Alpha-1-antichymotrypsin	EIGELYLPK	NO	YES	YES	YES
Alpha-1-antitrypsin	LSITGTYDLK	YES	YES	NO	NO
Alpha-2-antiplasmin	LGNQEPGGQTALK	YES	YES	YES	YES
Alpha-2-macroglobulin	AIGYLNTGYQR	YES	YES	YES	YES
Antithrombin-III	DDLYVSDAFHK	YES	NO	NO	NO
Apolipoprotein A-I	ATEHLSTLSEK	YES	YES	YES	YES
Apolipoprotein A-IV	LGEVNTYAGDLQK	YES	YES	YES	YES
Apolipoprotein B-100	FPEVDVLTK	YES	YES	YES	YES
Apolipoprotein E	LGPLVEQGR	YES	YES	YES	YES
Attractin	SVNNVVVR	YES	NO	YES	YES
Beta-2-glycoprotein 1	ATVVYQGER	YES	YES	YES	YES
Carbonic anhydrase 1	VLDALQAIK	YES	YES	YES	YES
CD5 antigen-like	LVGGLHR	YES	YES	NO	YES
Clusterin	ELDESLQVAER	YES	YES	YES	YES
Complement C1r subcomponent	GLTLHLK	YES	YES	NO	YES
Complement C3	TGLQEVEVK	YES	YES	YES	YES
Complement component C9	LSPIYNLVPVK	YES	YES	YES	YES
Complement factor B	EELLPAQDIK	YES	YES	NO	YES
Fibrinogen gamma chain	YEASILTHDSSIR	YES	YES	YES	NO
Fibulin-1	TGYYFDGISR	YES	YES	YES	YES
Haptoglobin	DIAPTLTLYVGK	YES	YES	YES	NO
Hemoglobin subunit alpha	VGAHAGEYGAEALER	YES	YES	YES	YES
Hemopexin	NFPSPVDAAFR	YES	YES	YES	YES
Heparin cofactor 2	TLEAQLTPR	YES	YES	YES	YES
Hyaluronan-binding protein 2	VVLGDQDLK	YES	YES	YES	YES
Inter-alpha-trypsin inhibitor heavy chain H2	SLAPTAAAK	YES	YES	YES	YES
Kininogen-1	TVGSDTFYSFK	YES	YES	YES	YES
Pigment epithelium-derived factor	LQSLFDSPDFSK	YES	YES	YES	YES
Plasminogen	LFLEPTR	YES	YES	YES	YES
Serotransferrin	DGAGDVAFVK	YES	YES	YES	YES
Serum albumin	LVNEVTEFAK	YES	YES	YES	NO
Vitronectin	FEDGVLDPDYPR	YES	YES	YES	YES

Table 4. Proficiency testing of each laboratory for the uncorrelated proteins in Sample A. (NA = peptide detected but ratio value not available, NP = not performed, 0 = peptide not detected).

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Adiponectin	0.098	0.111	0.014	0.209	0.098	NP	0.098	0.098	0.098	0.01	0.088	0.108	0.098	0.09	0.004	0.191
Afamin	0.063	0.065	0.002	0.128	0.063	NP	0.063	0.063	0.063	0	0.063	0.063	0.063	0.07	0.009	0.135
Alpha-1-acid glycoprotein 1	0.042	0.049	0.007	0.091	0.042	NP	0.042	0.042	0.042	NA	0.042	0.042	0.042	0.12	0.075	0.158
Alpha-1-antichymotrypsin	0.049	0	0.049	0.049	0.049	NP	0.049	0.049	0.049	0	0.049	0.049	0.049	0.06	0.010	0.107
Alpha-2-antiplasmin	2.054	2.083	0.029	4.137	2.054	NP	2.054	2.054	2.054	0.057	1.997	2.111	2.054	2.00	0.052	4.056
Alpha-2-macroglobulin	0.133	0.132	0.001	0.265	0.133	NP	0.133	0.133	0.133	0.01	0.123	0.143	0.133	0.15	0.017	0.282
Apolipoprotein A-I	0.240	0.230	0.010	0.470	0.240	NP	0.240	0.240	0.240	0.01	0.230	0.250	0.240	0.28	0.038	0.518
Apolipoprotein A-IV	2.480	2.351	0.128	4.831	2.480	NP	2.480	2.480	2.480	0.061	2.419	2.541	2.480	NA	2.480	2.480
Apolipoprotein B-100	1.650	1.645	0.006	3.295	1.650	NP	1.650	1.650	1.650	0.043	1.607	1.693	1.650	1.57	0.083	3.218
Apolipoprotein E	0.372	0.400	0.028	0.773	0.372	NP	0.372	0.372	0.372	NA	0.372	0.372	0.372	0.41	0.040	0.785
Attractin	1.261	1.249	0.012	2.510	1.261	NP	1.261	1.261	1.261	0	1.261	1.261	1.261	1.24	0.023	2.499
Beta-2-glycoprotein 1	1.317	1.221	0.096	2.538	1.317	NP	1.317	1.317	1.317	0.03	1.287	1.347	1.317	1.16	0.159	2.475
Biotinidase	0.046	0.073	0.027	0.119	0.046	NP	0.046	0.046	0.046	0	0.046	0.046	0.046	0	0.046	0.046
Carbonic anhydrase 1	1.304	1.233	0.071	2.537	1.304	NP	1.304	1.304	1.304	0	1.304	1.304	1.304	0.66	0.639	1.968
CD5 antigen-like	0.044	0.047	0.002	0.091	0.044	NP	0.044	0.044	0.044	0	0.044	0.044	0.044	0.05	0.003	0.092
Cholinesterase	0.080	0.076	0.004	0.156	0.080	NP	0.080	0.080	0.080	0.01	0.070	0.090	0.080	0	0.080	0.080
Clusterin	0.039	0.040	0.001	0.079	0.039	NP	0.039	0.039	0.039	NA	0.039	0.039	0.039	0.14	0.105	0.182
Coagulation factor XII	1.533	1.544	0.011	3.077	1.533	NP	1.533	1.533	1.533	0	1.533	1.533	1.533	0	1.533	1.533
Complement C1r subcomponent	0.090	0.100	0.010	0.190	0.090	NP	0.090	0.090	0.090	0	0.090	0.090	0.090	0.16	0.065	0.245
Complement C3	0.301	0.335	0.034	0.636	0.301	NP	0.301	0.301	0.301	0.01	0.291	0.311	0.301	0.31	0.007	0.609
Complement component C9	0.045	0.047	0.003	0.092	0.045	NP	0.045	0.045	0.045	0.01	0.035	0.055	0.045	0.06	0.018	0.107
Complement factor B	0.035	0.038	0.004	0.073	0.035	NP	0.035	0.035	0.035	0	0.035	0.035	0.035	0.04	0.010	0.080
Fibulin-1	0.043	0.043	0.001	0.086	0.043	NP	0.043	0.043	0.043	0	0.043	0.043	0.043	0.04	0.000	0.087
Hemoglobin subunit alpha	0.070	0.052	0.018	0.122	0.070	NP	0.070	0.070	0.070	0	0.070	0.070	0.070	0.69	0.617	0.757
Hemopexin	0.979	0.944	0.035	1.923	0.979	NP	0.979	0.979	0.979	0.029	0.950	1.008	0.979	1.01	0.031	1.989
Heparin cofactor 2	0.034	0.038	0.004	0.072	0.034	NP	0.034	0.034	0.034	0.01	0.024	0.044	0.034	NA	0.034	0.034
Hyaluronan-binding protein 2	0.058	0.073	0.015	0.131	0.058	NP	0.058	0.058	0.058	0.01	0.048	0.068	0.058	0.23	0.172	0.288

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Inter-alpha-trypsin inhibitor heavy chain H2	2.551	2.839	0.288	5.390	2.551	NP	2.551	2.551	2.551	0.062	2.489	2.613	2.551	2.44	0.113	4.989
Kininogen-1	0.188	0.176	0.012	0.364	0.188	NP	0.188	0.188	0.188	0.01	0.178	0.198	0.188	0.20	0.014	0.390
Pigment epithelium-derived factor	0.156	0.180	0.023	0.336	0.156	NP	0.156	0.156	0.156	0.01	0.146	0.166	0.156	0.18	0.027	0.340
Plasma protease C1 inhibitor	0.683	0.633	0.051	1.316	0.683	NP	0.683	0.683	0.683	0.017	0.666	0.700	0.683	0	0.683	0.683
Plasminogen	1.200	1.185	0.015	2.385	1.200	NP	1.200	1.200	1.200	0	1.200	1.200	1.200	1.26	0.055	2.455
Prothrombin	0.446	0.554	0.108	1.000	0.446	NP	0.446	0.446	0.446	0.06	0.386	0.506	0.446	0	0.446	0.446
Serotransferrin	0.215	0.259	0.044	0.474	0.215	NP	0.215	0.215	0.215	0.01	0.205	0.225	0.215	0.23	0.011	0.441
Vitronectin	0.434	0.516	0.082	0.950	0.434	NP	0.434	0.434	0.434	0.012	0.422	0.446	0.434	0.49	0.054	0.922
Peptides detected	**35**	**34**			**35**	**NP**			**35**	**23**			**35**	**30**		
Sum			**1.25**	**40.89**			**20.33**	**20.33**			**19.85**	**20.81**			**7.75**	**35.67**
Similarity (peptide identification)	**0.97**				**NP**				**0.66**				**0.86**			
Dissimilarity (peptide ratio identification)	**0.03**				**NP**				**0.95**				**0.22**			

Table 5. Proficiency testing of each laboratory for the uncorrelated proteins in Sample B. (NA = peptide detected but ratio value not available, NP = not performed, 0 = peptide not detected).

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Adiponectin	0.865	0.852	0.013	1.717	0.865	NP	0.865	0.865	0.865	0.016	0.849	0.881	0.865	0.10	0.770	0.960
Afamin	0.476	0.450	0.026	0.926	0.476	NP	0.476	0.476	0.476	0	0.476	0.476	0.476	0.48	0.008	0.960
Alpha-1-acid glycoprotein 1	0.625	0.661	0.036	1.285	0.625	NP	0.625	0.625	0.625	NA	0.625	0.625	0.625	0.68	0.053	1.302
Alpha-1-antichymotrypsin	0.367	0	0.367	0.367	0.367	NP	0.367	0.367	0.367	0	0.367	0.367	0.367	0.34	0.027	0.707
Alpha-2-antiplasmin	0.057	0.066	0.009	0.123	0.057	NP	0.057	0.057	0.057	0.01	0.047	0.067	0.057	0.07	0.015	0.129
Alpha-2-macroglobulin	0.024	0.028	0.004	0.053	0.024	NP	0.024	0.024	0.024	0.01	0.014	0.034	0.024	0.03	0.006	0.055
Apolipoprotein A-I	0.065	0.063	0.002	0.127	0.065	NP	0.065	0.065	0.065	0.01	0.055	0.075	0.065	0.08	0.011	0.140
Apolipoprotein A-IV	0.346	0.360	0.014	0.706	0.346	NP	0.346	0.346	0.346	0.014	0.332	0.360	0.346	NA	0.346	0.346
Apolipoprotein B-100	0.038	0.130	0.092	0.167	0.038	NP	0.038	0.038	0.038	0.01	0.028	0.048	0.038	0.07	0.028	0.103
Apolipoprotein E	0.055	0.063	0.008	0.118	0.055	NP	0.055	0.055	0.055	NA	0.055	0.055	0.055	0.09	0.035	0.145
Attractin	0.153	0.165	0.012	0.318	0.153	NP	0.153	0.153	0.153	0	0.153	0.153	0.153	NA	0.153	0.153
Beta-2-glycoprotein 1	0.228	0.221	0.007	0.449	0.228	NP	0.228	0.228	0.228	0.01	0.218	0.238	0.228	0.21	0.020	0.435
Biotinidase	0.082	0.108	0.026	0.190	0.082	NP	0.082	0.082	0.082	0	0.082	0.082	0.082	0	0.082	0.082
Carbonic anhydrase 1	0.325	0.323	0.003	0.648	0.325	NP	0.325	0.325	0.325	0	0.325	0.325	0.325	0.25	0.073	0.578
CD5 antigen-like	0.185	0.196	0.012	0.381	0.185	NP	0.185	0.185	0.185	0	0.185	0.185	0.185	0.20	0.013	0.382
Cholinesterase	1.222	0.969	0.253	2.191	1.222	NP	1.222	1.222	1.222	0.031	1.191	1.253	1.222	0	1.222	1.222
Clusterin	1.669	1.636	0.033	3.305	1.669	NP	1.669	1.669	1.669	NA	1.669	1.669	1.669	1.67	0.004	3.334
Coagulation factor XII	0.264	0.287	0.023	0.551	0.264	NP	0.264	0.264	0.264	0	0.264	0.264	0.264	0	0.264	0.264
Complement C1r subcomponent	0.660	0.685	0.025	1.345	0.660	NP	0.660	0.660	0.660	0	0.660	0.660	0.660	0.63	0.028	1.292
Complement C3	1.692	1.665	0.027	3.356	1.692	NP	1.692	1.692	1.692	0.044	1.648	1.736	1.692	1.44	0.252	3.131
Complement component C9	1.882	1.752	0.130	3.633	1.882	NP	1.882	1.882	1.882	0.044	1.838	1.926	1.882	1.83	0.047	3.716
Complement factor B	0.184	0.189	0.005	0.373	0.184	NP	0.184	0.184	0.184	0	0.184	0.184	0.184	0.18	0.006	0.362
Fibulin-1	0.706	0.662	0.045	1.368	0.706	NP	0.706	0.706	0.706	0	0.706	0.706	0.706	0.68	0.022	1.390
Hemoglobin subunit alpha	1.420	1.281	0.139	2.701	1.420	NP	1.420	1.420	1.420	0	1.420	1.420	1.420	0.58	0.843	1.997
Hemopexin	0.044	0.051	0.007	0.095	0.044	NP	0.044	0.044	0.044	0.01	0.034	0.054	0.044	0.07	0.026	0.113
Heparin cofactor 2	0.263	0.254	0.009	0.517	0.263	NP	0.263	0.263	0.263	0.011	0.252	0.274	0.263	NA	0.263	0.263
Hyaluronan-binding protein 2	0.537	0.588	0.052	1.125	0.537	NP	0.537	0.537	0.537	0.017	0.520	0.554	0.537	0.86	0.319	1.392

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Inter-alpha-trypsin inhibitor heavy chain H2	0.329	0.240	0.090	0.569	0.329	NP	0.329	0.329	0.329	0.01	0.319	0.339	0.329	0.32	0.007	0.651
Kininogen-1	0.036	0.038	0.002	0.074	0.036	NP	0.036	0.036	0.036	0.01	0.026	0.046	0.036	0.05	0.011	0.083
Pigment epithelium-derived factor	1.053	1.000	0.054	2.053	1.053	NP	1.053	1.053	1.053	0.029	1.024	1.082	1.053	1.02	0.037	2.070
Plasma protease C1 inhibitor	0.014	0.018	0.005	0.032	0.014	NP	0.014	0.014	0.014	0.01	0.004	0.024	0.014	0	0.014	0.014
Plasminogen	0.031	0.043	0.011	0.074	0.031	NP	0.031	0.031	0.031	0	0.031	0.031	0.031	0.16	0.126	0.189
Prothrombin	0.129	0.181	0.051	0.310	0.129	NP	0.129	0.129	0.129	0.02	0.109	0.149	0.129	0	0.129	0.129
Serotransferrin	0.058	0.070	0.013	0.128	0.058	NP	0.058	0.058	0.058	0.01	0.048	0.068	0.058	0.07	0.008	0.123
Vitronectin	2.219	2.425	0.205	4.644	2.219	NP	2.219	2.219	2.219	0.061	2.158	2.280	2.219	2.22	0.001	4.440
Peptides detected	**35**	**34**			**35**	**NP**			**35**	**23**			**35**	**30**		
Sum			**1.81**	**36.02**			**18.30**	**18.30**			**17.92**	**18.69**			**5.27**	**32.65**
Similarity (peptide identification)	**0.97**				**NP**				**0.66**				**0.86**			
Dissimilarity (peptide ratio identification)	**0.05**				**NP**				**0.96**				**0.16**			

Table 6. Proficiency testing of each laboratory for the uncorrelated proteins in Sample C. (NA = peptide detected but ratio value not available, NP = not performed, 0 = peptide not detected).

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Adiponectin	0.026	0.034	0.008	0.060	0.026	NP	0.026	0.026	0.026	0.01	0.016	0.036	0.026	0.14	0.109	0.161
Afamin	0.018	0.020	0.002	0.038	0.018	NP	0.018	0.018	0.018	0	0.018	0.018	0.018	0.02	0.006	0.042
Alpha-1-acid glycoprotein 1	0.005	0.011	0.006	0.017	0.005	NP	0.005	0.005	0.005	NA	0.005	0.005	0.005	0.06	0.056	0.067
Alpha-1-antichymotrypsin	1.422	0	1.422	1.422	1.422	NP	1.422	1.422	1.422	0	1.422	1.422	1.422	1.25	0.177	2.667
Alpha-2-antiplasmin	0.210	0.198	0.012	0.408	0.210	NP	0.210	0.210	0.210	0.01	0.200	0.220	0.210	0.21	0.005	0.424
Alpha-2-macroglobulin	0.724	0.842	0.118	1.567	0.724	NP	0.724	0.724	0.724	0.02	0.704	0.744	0.724	0.74	0.012	1.460
Apolipoprotein A-I	1.695	1.617	0.078	3.312	1.695	NP	1.695	1.695	1.695	0.042	1.653	1.737	1.695	1.51	0.186	3.204
Apolipoprotein A-IV	0.065	0.044	0.021	0.109	0.065	NP	0.065	0.065	0.065	0.01	0.055	0.075	0.065	NA	0.065	0.065
Apolipoprotein B-100	0.217	0.247	0.030	0.464	0.217	NP	0.217	0.217	0.217	0.01	0.207	0.227	0.217	0.25	0.036	0.470
Apolipoprotein E	2.356	2.280	0.076	4.636	2.356	NP	2.356	2.356	2.356	NA	2.356	2.356	2.356	2.22	0.135	4.577
Attractin	0.060	0.064	0.005	0.124	0.060	NP	0.060	0.060	0.060	0	0.060	0.060	0.060	NA	0.060	0.060
Beta-2-glycoprotein 1	0.052	0.052	0.000	0.104	0.052	NP	0.052	0.052	0.052	0.01	0.042	0.062	0.052	0.05	0.001	0.103
Biotinidase	0.385	0.457	0.072	0.842	0.385	NP	0.385	0.385	0.385	0	0.385	0.385	0.385	0	0.385	0.385
Carbonic anhydrase 1	0.043	0.059	0.016	0.102	0.043	NP	0.043	0.043	0.043	0	0.043	0.043	0.043	0.11	0.069	0.154
CD5 antigen-like	1.348	1.361	0.013	2.709	1.348	NP	1.348	1.348	1.348	0	1.348	1.348	1.348	1.42	0.075	2.771
Cholinesterase	0.163	0.198	0.035	0.361	0.163	NP	0.163	0.163	0.163	0.01	0.153	0.173	0.163	0	0.163	0.163
Clusterin	0.201	0.227	0.026	0.429	0.201	NP	0.201	0.201	0.201	NA	0.201	0.201	0.201	0.35	0.146	0.549
Coagulation factor XII	0.034	0.050	0.016	0.084	0.034	NP	0.034	0.034	0.034	0	0.034	0.034	0.034	0	0.034	0.034
Complement C1r subcomponent	0.019	0.040	0.021	0.059	0.019	NP	0.019	0.019	0.019	0	0.019	0.019	0.019	0.07	0.054	0.093
Complement C3	0.040	0.048	0.008	0.088	0.040	NP	0.040	0.040	0.040	0.01	0.030	0.050	0.040	0.05	0.012	0.092
Complement component C9	0.279	0.273	0.005	0.552	0.279	NP	0.279	0.279	0.279	0.01	0.269	0.289	0.279	0.30	0.017	0.574
Complement factor B	1.178	1.154	0.024	2.332	1.178	NP	1.178	1.178	1.178	0	1.178	1.178	1.178	1.05	0.126	2.230
Fibulin-1	0.144	0.132	0.012	0.275	0.144	NP	0.144	0.144	0.144	0	0.144	0.144	0.144	0.15	0.007	0.294
Hemoglobin subunit alpha	0.212	0.285	0.073	0.497	0.212	NP	0.212	0.212	0.212	0	0.212	0.212	0.212	0.91	0.703	1.127
Hemopexin	0.274	0.281	0.007	0.555	0.274	NP	0.274	0.274	0.274	0.01	0.264	0.284	0.274	0.31	0.032	0.581
Heparin cofactor 2	1.892	1.775	0.117	3.667	1.892	NP	1.892	1.892	1.892	0.051	1.841	1.943	1.892	NA	1.892	1.892
Hyaluronan-binding protein 2	0.123	0.136	0.013	0.260	0.123	NP	0.123	0.123	0.123	0.01	0.113	0.133	0.123	0.48	0.352	0.598
Inter-alpha-trypsin inhibitor heavy chain H2	0.062	0.083	0.021	0.145	0.062	NP	0.062	0.062	0.062	0.01	0.052	0.072	0.062	0.07	0.013	0.137

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Kininogen-1	1.129	NA	1.129	1.129	1.129	NP	1.129	1.129	1.129	0.032	1.097	1.161	1.129	1.11	0.020	2.237
Pigment epithelium-derived factor	0.021	0.023	0.002	0.045	0.021	NP	0.021	0.021	0.021	0.01	0.011	0.031	0.021	0.03	0.013	0.056
Plasma protease C1 inhibitor	0.093	0.106	0.013	0.199	0.093	NP	0.093	0.093	0.093	0.01	0.083	0.103	0.093	0	0.093	0.093
Plasminogen	0.148	0.179	0.031	0.327	0.148	NP	0.148	0.148	0.148	0	0.148	0.148	0.148	0.19	0.038	0.333
Prothrombin	0.040	0.053	0.013	0.094	0.040	NP	0.040	0.040	0.040	0.01	0.030	0.050	0.040	0	0.040	0.040
Serotransferrin	1.360	1.206	0.154	2.566	1.360	NP	1.360	1.360	1.360	0.036	1.324	1.396	1.360	1.29	0.066	2.654
Vitronectin	0.068	0.089	0.021	0.158	0.068	NP	0.068	0.068	0.068	0.01	0.058	0.078	0.068	0.10	0.032	0.169
Peptides detected	**35**	**34**			**35**	**NP**			**35**	**23**			**35**	**30**		
Sum			**3.62**	**29.73**			**16.11**	**16.11**			**15.78**	**16.44**			**5.23**	**30.56**
Similarity (peptide identification)	**0.97**				**NP**				**0.66**				**0.86**			
Dissimilarity (peptide ratio identification)	**0.12**				**NP**				**0.96**				**0.17**			

Table 7. Proficiency testing of each laboratory for the correlated proteins in Sample D. (NA = peptide detected but ratio value not available, 0 = peptide not detected).

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Adiponectin	0.084	0.089	0.005	0.173	0.084	0.11	0.026	0.194	0.084	0.088	0.004	0.172	0.084	0.16	0.079	0.247
Afamin	0.115	0.115	0.001	0.230	0.115	0.12	0.005	0.235	0.115	0.118	0.003	0.233	0.115	0.12	0.010	0.239
Alpha-1-acid glycoprotein 1	0.196	0.209	0.014	0.405	0.196	0.23	0.034	0.426	0.196	NA	0.196	0.196	0.196	0.25	0.058	0.450
Alpha-1-antichymotrypsin	0.342	0	0.342	0.342	0.342	0.36	0.018	0.702	0.342	0.399	0.057	0.741	0.342	0.29	0.055	0.629
Alpha-1-antitrypsin	0.168	0.166	0.002	0.333	0.168	0.17	0.002	0.338	0.168	0	0.168	0.168	0.168	0	0.168	0.168
Alpha-2-antiplasmin	0.265	0.279	0.014	0.544	0.265	0.28	0.015	0.545	0.265	0.296	0.031	0.561	0.265	0.28	0.013	0.543
Alpha-2-macroglobulin	0.128	0.140	0.011	0.268	0.128	0.15	0.022	0.278	0.128	0.161	0.033	0.289	0.128	0.14	0.011	0.268
Antithrombin-III	0.016	0.020	0.003	0.036	0.016	0	0.016	0.016	0.016	0	0.016	0.016	0.016	0	0.016	0.016
Apolipoprotein A-I	0.232	0.248	0.016	0.480	0.232	0.24	0.008	0.472	0.232	0.273	0.041	0.505	0.232	0.25	0.020	0.484
Apolipoprotein A-IV	0.300	0.312	0.013	0.612	0.300	0.3	0.000	0.600	0.300	0.351	0.051	0.651	0.300	NA	0.300	0.300
Apolipoprotein B-100	0.337	0.338	0.001	0.675	0.337	0.36	0.023	0.697	0.337	0.378	0.041	0.715	0.337	0.35	0.015	0.689
Apolipoprotein E	0.300	0.320	0.020	0.620	0.300	0.33	0.030	0.630	0.300	0.299	0.001	0.599	0.300	0.33	0.027	0.627
Attractin	0.155	0.177	0.022	0.331	0.155	0	0.155	0.155	0.155	NA	0.155	0.155	0.155	0.19	0.033	0.342
Beta-2-glycoprotein 1	0.312	0.328	0.016	0.639	0.312	0.36	0.048	0.672	0.312	0.355	0.043	0.667	0.312	0.34	0.026	0.650
Carbonic anhydrase 1	0.228	0.246	0.018	0.473	0.228	0.26	0.032	0.488	0.228	0.246	0.018	0.474	0.228	0.24	0.016	0.471
CD5 antigen-like	0.164	0.179	0.016	0.343	0.164	0.19	0.026	0.354	0.164	0	0.164	0.164	0.164	0.19	0.024	0.352
Clusterin	0.083	0.095	0.012	0.178	0.083	0.11	0.027	0.193	0.083	0.095	0.012	0.178	0.083	0.10	0.020	0.187
Complement C1r subcomponent	0.089	0.108	0.019	0.197	0.089	0.12	0.031	0.209	0.089	0	0.089	0.089	0.089	0.08	0.004	0.173
Complement C3	0.238	0.240	0.001	0.478	0.238	0.25	0.012	0.488	0.238	0.278	0.040	0.516	0.238	0.23	0.008	0.469
Complement component C9	0.196	0.200	0.005	0.396	0.196	0.23	0.034	0.426	0.196	0.214	0.018	0.410	0.196	0.21	0.017	0.408
Complement factor B	0.257	0.260	0.003	0.518	0.257	0.83	0.573	1.087	0.257	0	0.257	0.257	0.257	0.24	0.013	0.502
Fibrinogen gamma chain	0.152	0.172	0.020	0.324	0.152	0.18	0.028	0.332	0.152	0.178	0.026	0.330	0.152	0	0.152	0.152
Fibulin-1	0.105	0.116	0.012	0.221	0.105	0.12	0.015	0.225	0.105	0.094	0.011	0.199	0.105	0.11	0.007	0.217
Haptoglobin	0.166	0.169	0.003	0.336	0.166	0.22	0.054	0.386	0.166	0.199	0.033	0.365	0.166	0	0.166	0.166
Hemoglobin subunit alpha	0.288	0.297	0.008	0.585	0.288	0.31	0.022	0.598	0.288	0.268	0.020	0.556	0.288	1.10	0.807	1.384
Hemopexin	0.200	0.205	0.005	0.405	0.200	0.23	0.030	0.430	0.200	0.225	0.025	0.425	0.200	0.23	0.033	0.432
Heparin cofactor 2	0.266	0.270	0.004	0.535	0.266	0.26	0.006	0.526	0.266	0.274	0.008	0.540	0.266	0.27	0.000	0.532
Hyaluronan-binding protein 2	0.134	0.137	0.003	0.270	0.134	0.13	0.004	0.264	0.134	0.136	0.002	0.270	0.134	0.17	0.037	0.304

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Inter-alpha-trypsin inhibitor heavy chain H2	0.361	0.370	0.009	0.731	0.361	0.37	0.009	0.731	0.361	0.385	0.024	0.746	0.361	0.36	0.000	0.722
Kininogen-1	0.123	0.118	0.004	0.241	0.123	0.13	0.008	0.253	0.123	0.141	0.019	0.264	0.123	0.13	0.004	0.249
Pigment epithelium-derived factor	0.128	0.132	0.004	0.260	0.128	0.14	0.012	0.268	0.128	0.137	0.009	0.265	0.128	0.14	0.010	0.266
Plasminogen	0.179	0.199	0.020	0.378	0.179	0.23	0.051	0.409	0.179	NA	0.179	0.179	0.179	0.17	0.008	0.349
Serotransferrin	0.247	0.243	0.004	0.490	0.247	0.27	0.023	0.517	0.247	0.247	0.000	0.494	0.247	0.26	0.016	0.510
Serum albumin	0.242	0.232	0.010	0.474	0.242	0.27	0.028	0.512	0.242	1.43	1.188	1.672	0.242	0	0.242	0.242
Vitronectin	0.600	0.633	0.032	1.233	0.600	0.6	0.000	1.200	0.600	0.582	0.018	1.182	0.600	0.59	0.013	1.188
Peptides detected	**35**	**34**			**35**	**33**			**35**	**30**			**35**	**30**		
Sum			**0.69**	**14.75**			**1.43**	**15.85**			**3.0**	**15.24**			**2.43**	**14.93**
Similarity (peptide identification)	**0.97**				**0.94**				**0.86**				**0.86**			
Dissimilarity (peptide ratio identification)	**0.05**				**0.09**				**0.19**				**0.16**			

Table 8. Proficiency testing of each laboratory for the correlated proteins in Sample E. (NA = peptide detected but ratio value not available, 0 = peptide not detected).

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Adiponectin	0.538	0.541	0.002	1.079	0.538	0.57	0.032	1.108	0.538	0.259	0.279	0.797	0.538	0.89	0.352	1.429
Afamin	0.705	0.674	0.031	1.379	0.705	0.69	0.015	1.395	0.705	0.335	0.370	1.040	0.705	0.94	0.237	1.647
Alpha-1-acid glycoprotein 1	1.189	1.266	0.077	2.455	1.189	1.34	0.151	2.529	1.189	NA	1.189	1.189	1.189	2.01	0.824	3.202
Alpha-1-antichymotrypsin	2.050	0.000	2.050	2.050	2.050	1.98	0.070	4.030	2.050	0.938	1.112	2.988	2.050	2.34	0.291	4.392
Alpha-1-antitrypsin	0.989	0.975	0.014	1.964	0.989	1.01	0.021	1.999	0.989	0	0.989	0.989	0.989	0	0.989	0.989
Alpha-2-antiplasmin	1.550	1.633	0.083	3.182	1.550	1.6	0.051	3.150	1.550	0.8	0.750	2.350	1.550	2.16	0.615	3.714
Alpha-2-macroglobulin	0.797	0.799	0.002	1.596	0.797	0.85	0.053	1.647	0.797	0.393	0.404	1.190	0.797	1.15	0.353	1.947
Antithrombin-III	0.100	0.111	0.011	0.211	0.100	0	0.100	0.100	0.100	0	0.100	0.100	0.100	0	0.100	0.100
Apolipoprotein A-I	1.448	1.429	0.019	2.877	1.448	1.42	0.028	2.868	1.448	0.755	0.693	2.203	1.448	2.07	0.618	3.514
Apolipoprotein A-IV	1.864	1.915	0.051	3.780	1.864	1.75	0.114	3.614	1.864	0.852	1.012	2.716	1.864	NA	1.864	1.864
Apolipoprotein B-100	2.055	1.913	0.142	3.969	2.055	1.99	0.065	4.045	2.055	0.953	1.102	3.008	2.055	3.35	1.299	5.409
Apolipoprotein E	1.841	1.794	0.047	3.635	1.841	1.88	0.039	3.721	1.841	0.925	0.916	2.766	1.841	2.63	0.790	4.472
Attractin	0.967	0.991	0.024	1.958	0.967	0	0.967	0.967	0.967	NA	0.967	0.967	0.967	1.44	0.469	2.403
Beta-2-glycoprotein 1	1.959	1.880	0.079	3.839	1.959	1.93	0.029	3.889	1.959	0.957	1.002	2.916	1.959	2.60	0.642	4.560
Carbonic anhydrase 1	1.497	1.444	0.053	2.942	1.497	1.41	0.087	2.907	1.497	0.704	0.793	2.201	1.497	1.89	0.388	3.383
CD5 antigen-like	0.957	1.039	0.082	1.997	0.957	1.16	0.203	2.117	0.957	0	0.957	0.957	0.957	1.43	0.471	2.386
Clusterin	0.546	0.552	0.006	1.098	0.546	0.58	0.034	1.126	0.546	0.302	0.244	0.848	0.546	0.87	0.321	1.413
Complement C1r subcomponent	0.575	0.650	0.075	1.226	0.575	0.61	0.035	1.185	0.575	0	0.575	0.575	0.575	0.72	0.145	1.295
Complement C3	1.411	1.387	0.024	2.798	1.411	1.35	0.061	2.761	1.411	0.739	0.672	2.150	1.411	1.83	0.415	3.238
Complement component C9	1.155	1.136	0.019	2.291	1.155	1.2	0.045	2.355	1.155	0.559	0.596	1.714	1.155	1.80	0.641	2.952
Complement factor B	1.577	1.491	0.086	3.068	1.577	2.06	0.483	3.637	1.577	0	1.577	1.577	1.577	2.04	0.464	3.618
Fibrinogen gamma chain	0.967	0.978	0.011	1.945	0.967	0.97	0.003	1.937	0.967	0.48	0.487	1.447	0.967	0	0.967	0.967
Fibulin-1	0.648	0.640	0.008	1.288	0.648	0.66	0.012	1.308	0.648	0.363	0.285	1.011	0.648	0.94	0.296	1.592
Haptoglobin	1.034	0.992	0.042	2.026	1.034	1.07	0.036	2.104	1.034	0.532	0.502	1.566	1.034	0	1.034	1.034
Hemoglobin subunit alpha	1.770	1.716	0.054	3.486	1.770	1.78	0.010	3.550	1.770	0.933	0.837	2.703	1.770	1.07	0.696	2.844
Hemopexin	1.214	1.192	0.023	2.406	1.214	1.21	0.004	2.424	1.214	0.586	0.628	1.800	1.214	1.79	0.573	3.002
Heparin cofactor 2	1.631	1.536	0.095	3.166	1.631	1.38	0.251	3.011	1.631	0.771	0.860	2.402	1.631	2.09	0.462	3.723
Hyaluronan-binding protein 2	0.845	0.815	0.029	1.660	0.845	0.75	0.095	1.595	0.845	0.408	0.437	1.253	0.845	1.12	0.271	1.961

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Inter-alpha-trypsin inhibitor heavy chain H2	2.191	1.969	0.221	4.160	2.191	2.08	0.111	4.271	2.191	0.998	1.193	3.189	2.191	2.88	0.693	5.075
Kininogen-1	0.736	0.728	0.008	1.464	0.736	0.73	0.006	1.466	0.736	0.364	0.372	1.100	0.736	1.03	0.289	1.761
Pigment epithelium-derived factor	0.773	0.765	0.008	1.538	0.773	0.8	0.027	1.573	0.773	0.379	0.394	1.152	0.773	1.16	0.386	1.931
Plasminogen	1.077	1.102	0.025	2.179	1.077	1.06	0.017	2.137	1.077	NA	1.077	1.077	1.077	1.24	0.162	2.316
Serotransferrin	1.500	1.483	0.016	2.983	1.500	1.44	0.060	2.940	1.500	0.717	0.783	2.217	1.500	2.02	0.516	3.516
Serum albumin	1.371	1.374	0.003	2.745	1.371	1.26	0.111	2.631	1.371	1.219	0.152	2.590	1.371	0	1.371	1.371
Vitronectin	3.643	3.627	0.016	7.270	3.643	3.29	0.353	6.933	3.643	1.941	1.702	5.584	3.643	5.43	1.785	9.071
Peptides detected	**35**	**34**			**35**	**33**			**35**	**30**			**35**	**30**		
Sum			**3.54**	**87.71**			**3.78**	**89.03**			**26.0**	**64.33**			**21.79**	**98.09**
Similarity (peptide identification)	**0.97**				**0.94**				**0.86**				**0.86**			
Dissimilarity (peptide ratio identification)	**0.04**				**0.04**				**0.4**				**0.22**			

Table 9. Proficiency testing of each laboratory for the correlated proteins in Sample F. (NA = peptide detected but ratio value not available, NP = not performed, 0 = peptide not detected).

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Adiponectin	3.012	NP	3.012	3.012	3.012	3.15	0.138	6.162	3.012	0.366	2.646	3.378	3.012	1.74	1.274	4.749
Afamin	3.956	NP	3.956	3.956	3.956	3.68	0.276	7.636	3.956	0.47	3.486	4.426	3.956	3.34	0.617	7.295
Alpha-1-acid glycoprotein 1	7.068	NP	7.068	7.068	7.068	6.72	0.348	13.788	7.068	NA	7.068	7.068	7.068	5.81	1.254	12.883
Alpha-1-antichymotrypsin	11.446	NP	11.446	11.446	11.446	8.7	2.746	20.146	11.446	1.394	10.052	12.840	11.446	8.37	3.072	19.819
Alpha-1-antitrypsin	5.734	NP	5.734	5.734	5.734	4.93	0.804	10.664	5.734	0	5.734	5.734	5.734	0	5.734	5.734
Alpha-2-antiplasmin	9.131	NP	9.131	9.131	9.131	7.3	1.831	16.431	9.131	1.116	8.015	10.247	9.131	8.11	1.019	17.244
Alpha-2-macroglobulin	4.489	NP	4.489	4.489	4.489	4.2	0.289	8.689	4.489	0.573	3.916	5.062	4.489	4.09	0.395	8.583
Antithrombin-III	0.601	NP	0.601	0.601	0.601	0	0.601	0.601	0.601	0	0.601	0.601	0.601	0	0.601	0.601
Apolipoprotein A-I	8.082	NP	8.082	8.082	8.082	7.15	0.932	15.232	8.082	0.974	7.108	9.056	8.082	7.15	0.935	15.228
Apolipoprotein A-IV	10.185	NP	10.185	10.185	10.185	9.63	0.555	19.815	10.185	1.349	8.836	11.534	10.185	NA	10.185	10.185
Apolipoprotein B-100	11.957	NP	11.957	11.957	11.957	10.77	1.187	22.727	11.957	1.537	10.420	13.494	11.957	9.65	2.305	21.608
Apolipoprotein E	10.649	NP	10.649	10.649	10.649	9.53	1.119	20.179	10.649	1.333	9.316	11.982	10.649	9.34	1.308	19.989
Attractin	5.296	NP	5.296	5.296	5.296	0	5.296	5.296	5.296	NA	5.296	5.296	5.296	4.98	0.319	10.274
Beta-2-glycoprotein 1	10.713	NP	10.713	10.713	10.713	9.84	0.873	20.553	10.713	1.401	9.312	12.114	10.713	9.10	1.609	19.816
Carbonic anhydrase 1	8.206	NP	8.206	8.206	8.206	7.35	0.856	15.556	8.206	0.987	7.219	9.193	8.206	6.87	1.340	15.072
CD5 antigen-like	5.667	NP	5.667	5.667	5.667	5.38	0.287	11.047	5.667	0	5.667	5.667	5.667	5.02	0.650	10.685
Clusterin	3.180	NP	3.180	3.180	3.180	3.23	0.050	6.410	3.180	0.39	2.790	3.570	3.180	2.65	0.530	5.829
Complement C1r subcomponent	3.299	NP	3.299	3.299	3.299	2.95	0.349	6.249	3.299	0	3.299	3.299	3.299	2.84	0.455	6.142
Complement C3	7.875	NP	7.875	7.875	7.875	7	0.875	14.875	7.875	1.03	6.845	8.905	7.875	6.72	1.157	14.592
Complement component C9	5.994	NP	5.994	5.994	5.994	5.12	0.874	11.114	5.994	0.829	5.165	6.823	5.994	6.07	0.071	12.059
Complement factor B	9.149	NP	9.149	9.149	9.149	7.95	1.199	17.099	9.149	0	9.149	9.149	9.149	7.05	2.099	16.199
Fibrinogen gamma chain	5.361	NP	5.361	5.361	5.361	5.31	0.051	10.671	5.361	0.844	4.517	6.205	5.361	0	5.361	5.361
Fibulin-1	3.705	NP	3.705	3.705	3.705	3.37	0.335	7.075	3.705	0.549	3.156	4.254	3.705	3.28	0.426	6.984
Haptoglobin	5.652	NP	5.652	5.652	5.652	5.97	0.318	11.622	5.652	0.782	4.870	6.434	5.652	0	5.652	5.652
Hemoglobin subunit alpha	9.957	NP	9.957	9.957	9.957	9.32	0.637	19.277	9.957	1.293	8.664	11.250	9.957	2.31	7.646	12.269
Hemopexin	6.889	NP	6.889	6.889	6.889	5.57	1.319	12.459	6.889	0.838	6.051	7.727	6.889	6.27	0.621	13.157
Heparin cofactor 2	8.838	NP	8.838	8.838	8.838	7.41	1.428	16.248	8.838	1.077	7.761	9.915	8.838	7.83	1.011	16.665
Hyaluronan-binding protein 2	4.928	NP	4.928	4.928	4.928	3.88	1.048	8.808	4.928	0.642	4.286	5.570	4.928	4.08	0.846	9.010

Proteins	Reference Ratio	Lab 1 Ratio	Difference	Sum	Reference Ratio	Lab 2 Ratio	Difference	Sum	Reference Ratio	Lab 3 Ratio	Difference	Sum	Reference Ratio	Lab 4 Ratio	Difference	Sum
Inter-alpha-trypsin inhibitor heavy chain H2	12.329	NP	12.329	12.329	12.329	10.6	1.729	22.929	12.329	1.415	10.914	13.744	12.329	10.29	2.035	22.624
Kininogen-1	4.013	NP	4.013	4.013	4.013	3.73	0.283	7.743	4.013	0.541	3.472	4.554	4.013	3.46	0.557	7.468
Pigment epithelium-derived factor	4.329	NP	4.329	4.329	4.329	4.2	0.129	8.529	4.329	0.549	3.780	4.878	4.329	3.94	0.394	8.264
Plasminogen	6.114	NP	6.114	6.114	6.114	6.44	0.326	12.554	6.114	NA	6.114	6.114	6.114	4.93	1.180	11.048
Serotransferrin	8.524	NP	8.524	8.524	8.524	6.43	2.094	14.954	8.524	1.011	7.513	9.535	8.524	7.31	1.217	15.832
Serum albumin	5.717	NP	5.717	5.717	5.717	4.23	1.487	9.947	5.717	1.117	4.600	6.834	5.717	0	5.717	5.717
Vitronectin	19.969	NP	19.969	19.969	19.969	16.84	3.129	36.809	19.969	2.444	17.525	22.413	19.969	16.59	3.377	36.561
Peptides detected	**35**	**NP**			**35**	**33**			**35**	**30**			**35**	**30**		
Sum			**252.01**	**252.01**			**35.80**	**469.89**			**225.16**	**278.86**			**72.97**	**431.20**
Similarity (peptide identification)	**NP**				**0.94**				**0.86**				**0.86**			
Dissimilarity (peptide ratio identification)	**NP**				**0.08**				**0.81**				**0.17**			

Table 10. Proficiency testing scores of each laboratory in comparison to the reference laboratory data for all samples analysed.

Sample	Laboratory	% Similarity (peptide identification)	% Dissimilarity (peptide ratio identification)
A	**Reference Laboratory**	**100**	**0**
A	Laboratory 1	97	3
A	Laboratory 2*	NA	NA
A	Laboratory 3	66	95
A	Laboratory 4	86	22
B	**Reference Laboratory**	**100**	**0**
B	Laboratory 1	97	5
B	Laboratory 2*	NA	NA
B	Laboratory 3	66	96
B	Laboratory 4	86	16
C	**Reference Laboratory**	**100**	**0**
C	Laboratory 1	97	12
C	Laboratory 2*	NA	NA
C	Laboratory 3	66	96
C	Laboratory 4	86	17
D	**Reference Laboratory**	**100**	**0**
D	Laboratory 1	97	5
D	Laboratory 2	94	9
D	Laboratory 3	86	19
D	Laboratory 4	86	16
E	**Reference Laboratory**	**100**	**0**
E	Laboratory 1	97	4
E	Laboratory 2	94	4
E	Laboratory 3	86	40
E	Laboratory 4	86	22
F	**Reference Laboratory**	**100**	**0**
F	Laboratory 1**	NA	NA
F	Laboratory 2	94	8
F	Laboratory 3	86	81
F	Laboratory 4	86	17

* NA (not assessed). Detection not applicable to Lab 2 as testing of the uncorrelated samples (A, B, C) could not be performed. Laboratory 2 were therefore not assessed for these samples.

** Laboratory 1 could not test Sample F and were not assessed for this sample.

Project findings

The use of both uncorrelated and correlated peptide standards was aimed at challenging laboratories for their ability to detect and identify each unknown peptide. Five laboratories were sent samples for analysis with four laboratories returning data. One laboratory did not perform any analyses due to unforeseen restructuring in their laboratory and therefore had to withdraw from the trial.

The data generated from this study indicates that protein diagnostics using human peptides is certainly possible. Laboratories appeared to detect the protein peptides in the correlated samples (D, E, F) with greater efficiency in comparison to the protein peptides in the uncorrelated samples (A, B, C) (Table 10). However, from laboratory feedback and from analysis of the laboratory generated data, two primary areas for improvement of an EQA program were identified. Firstly, the current EQA reference testing material needs to be improved since laboratories reported the same (or similar) problems encountered (see below). Secondly, there appears to be technical issues relating to the testing columns used for mass spectrometry given that some laboratories could not detect the unknown peptides. For example, Laboratory 2 were the only testing facility that could not perform any protein assay in the uncorrelated samples (A, B, C), and Laboratory 1 could not perform a protein assay for the correlated Sample F (see Table 10). However, the identification of these shortcomings is essential information to relay back to laboratories since it allows for the laboratory to troubleshoot their testing pipeline so that data analysis and performance can be improved. These data demonstrate the importance of participation in an EQA program. This study therefore achieved its aim in identifying areas of concern for laboratory testing and in highlighted technological shortcomings.

Problems encountered

Key problems were encountered in this study which resulted in a delay for data submission. A major hold-up was that each laboratory upon performing peptide measurements encountered difficulties in measuring several of the RCPAQAP reference testing peptides. There were subsequently multiple requests from laboratories for more samples to be sent so that a complete analysis could be made. Laboratories reported that upon measuring certain peptides, their mass spectrometer liquid chromatography peptide columns were damaged and had to be replaced from overseas suppliers. This was due to high pressures building up on the testing columns which resulted in blockages. This had an unforeseen impact in holding up each laboratory's diagnostic samples for clinical analyses. Subsequently, the RCPAQAP peptide samples were put on hold until the backlog of the clinical samples could be completed. Laboratories also reported issues relating to the quality of some samples since peptides could not be readily detected with consistent efficiency. However, these encountered

issues were precisely what this trial project was aimed at discovering given that this is a new potential area for human diagnostics.

Future diagnostics

(*Does the proposed project complement other similar services, activities and resources?*)

The data from this project directly complements another funded QUPP project. In 2017, QUPP funding (Agreement id: 4-4YYPT91) was awarded for developing EQA proficiency testing of, (i) DNA extraction, (ii) circulating free DNA for cancer-associated biomarkers and for non-invasive prenatal testing (NIPT), and (iii) leukaemia-associated DNA variants. The identification of protein biomarkers (that are potentially circulating in the blood) are therefore directly complementary. For example, future protein diagnostics may be used with DNA analyses to identify circulating levels of both proteins and DNA that are associated with different human diseases (Cohen et al 2017). Such analyses would be for more informative for the clinician and may help identify disease development early, so that appropriate clinical management can be initiated.

The development of future multi-protein biomarker EQA programs will also complement our existing programs. For example, we have molecular quality assurance programs in the area of inherited haematological disorders (i.e., haemochromatosis, thrombosis, and thalassaemia) and in the qualitative and quantitative detection of nucleic acids from infectious micro-organisms including viruses and bacteria. Quality assuring protein mass spectrometry will add disease-associated proteins/peptides to this EQA list.

Long term outcomes

(*Does the project provide value for money including: Economy, Efficiency and Effectiveness - delivering a better service or getting a better return for the same amount of expense, time or effort?*)

The potential for diagnostic applications using mass spectrometry is rapidly growing owing to the technology having increased levels of diagnostic sensitivity and high throughput capacity for testing. However, the technology has not yet been fully adopted for human genetic-associated disease testing partly due to the lack of quality assurance programs. This trial was therefore initiated to challenge mass spectrometer testing facilities to determine if disease-associated protein diagnostics is viable. The data produced from this study suggests that protein diagnostics using human peptides is possible. Thus, the long-term benefit of an EQA for mass spectrometry is that it will allow the RCPAQAP to develop proteomic EQA programs for (i) cancer (i.e., breast, lung, prostate gastrointestinal); (ii) for neurological disorders (i.e., Alzheimer, Parkinson, Huntington, epilepsy); and (iii) for age-related disease (i.e., cardiovascular disease). Importantly, proteomic results in combination with identified

genetic alterations will allow clinicians to make better informed decisions with respect to pharmaceutical intervention and patient management.

In terms of near future multi-protein biomarker testing, the development of quality assessments will allow diagnostic laboratories to detect protein variation or abnormal protein expression in a much shorter time-period with high accuracy and sensitivity. The cost of healthcare and treatment plans can therefore be significantly reduced and implemented earlier. Reduced patient invasiveness (due to only a blood sample being required) will increase patient care by allowing earlier diagnoses which will benefit patient management strategies and thus increase cost and time effectiveness.

The development of a proteomic EQA will allow the establishment of cross functional and inter-discipline collaboration. For example, the RCPAQAP Biosecurity, Serology and Microbiology disciplines will benefit from analyses of disease-associated protein targets. This combined discipline approach will result in the production of highly-developed quality assurance programs for future cost-effective analyses across the RCPAQAP.

The next step

The next step for the development of an RCPAQAP proteomic proficiency testing program is to perform a follow-on study that encompasses a larger cohort of laboratories. The challenges identified from this study need to be rectified and put in to practice before this EQA program can be offered for the proficiency testing of mass spectrometry. However, the current data will nonetheless allow us to improve on the development of a protein specific EQA. A larger cohort of data will additionally allow the RCPAQAP to apply for NATA accreditation. Importantly, the data from this study has been peer-reviewed and published (Horan et al., 2019).

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Appendix

Proteins and peptide sequences used in the reference testing samples.

Uncorrelated Protein	Peptide Sequence	Correlated Protein	Peptide Sequence
Adiponectin	IFYNQQNHYDGSTGK	Adiponectin	IFYNQQNHYDGSTGK
Afamin	DADPDTFFAK	Afamin	DADPDTFFAK
Alpha-1-acid glycoprotein 1	NWGLSVYADKPETTK	Alpha-1-acid glycoprotein 1	NWGLSVYADKPETTK
Alpha-1-antichymotrypsin	EIGELYLPK	Alpha-1-antichymotrypsin	EIGELYLPK
Alpha-2-antiplasmin	LGNQEPGGQTALK	Alpha-1-antitrypsin	LSITGTYDLK
Alpha-2-macroglobulin	AIGYLNTGYQR	Alpha-2-antiplasmin	LGNQEPGGQTALK
Apolipoprotein A-I	ATEHLSTLSEK	Alpha-2-macroglobulin	AIGYLNTGYQR
Apolipoprotein A-IV	LGEVNTYAGDLQK	Antithrombin-III	DDLYVSDAFHK
Apolipoprotein B-100	FPEVDVLTK	Apolipoprotein A-I	ATEHLSTLSEK
Apolipoprotein E	LGPLVEQGR	Apolipoprotein A-IV	LGEVNTYAGDLQK
Attractin	SVNNVVVR	Apolipoprotein B-100	FPEVDVLTK
Beta-2-glycoprotein 1	ATVVYQGER	Apolipoprotein E	LGPLVEQGR
Biotinidase	SHLIIAQVAK	Attractin	SVNNVVVR
Carbonic anhydrase 1	VLDALQAIK	Beta-2-glycoprotein 1	ATVVYQGER
CD5 antigen-like	LVGGLHR	Carbonic anhydrase 1	VLDALQAIK
Cholinesterase	YLTLNTESTR	CD5 antigen-like	LVGGLHR
Clusterin	ELDESLQVAER	Clusterin	ELDESLQVAER
Coagulation factor XII	EQPPSLTR	Complement C1r subcomponent	GLTLHLK
Complement C1r subcomponent	GLTLHLK	Complement C3	TGLQEVEVK
Complement C3	TGLQEVEVK	Complement component C9	LSPIYNLVPVK
Complement component C9	LSPIYNLVPVK	Complement factor B	EELLPAQDIK
Complement factor B	EELLPAQDIK	Fibrinogen gamma chain	YEASILTHDSSIR
Fibulin-1	TGYYFDGISR	Fibulin-1	TGYYFDGISR
Hemoglobin subunit alpha	VGAHAGEYGAEALER	Haptoglobin	DIAPTLTLYVGK
Hemopexin	NFPSPVDAAFR	Hemoglobin subunit alpha	VGAHAGEYGAEALER
Heparin cofactor 2	TLEAQLTPR	Hemopexin	NFPSPVDAAFR
Hyaluronan-binding protein 2	VVLGDQDLK	Heparin cofactor 2	TLEAQLTPR
Inter-alpha-trypsin inhibitor heavy chain H2	SLAPTAAAK	Hyaluronan-binding protein 2	VVLGDQDLK
Kininogen-1	TVGSDTFYSFK	Inter-alpha-trypsin inhibitor heavy chain H2	SLAPTAAAK
Pigment epithelium-derived factor	LQSLFDSPDFSK	Kininogen-1	TVGSDTFYSFK
Plasma protease C1 inhibitor	FQPTLLTLPR	Pigment epithelium-derived factor	LQSLFDSPDFSK
Plasminogen	LFLEPTR	Plasminogen	LFLEPTR
Prothrombin	ELLESYIDGR	Serotransferrin	DGAGDVAFVK
Serotransferrin	DGAGDVAFVK	Serum albumin	LVNEVTEFAK
Vitronectin	FEDGVLDPDYPR	Vitronectin	FEDGVLDPDYPR