NIHR | Cambridge Biomedical Research Centre

Core Biochemical Assay Laboratory (CBAL)

Users Handbook – 1st Edition June 2020

Cambridge University Hospitals NHS Foundation Trust

UNIVERSITY OF CAMBRIDGE

Foreword

The Core Biochemical Assay Laboratory was established in 2008 with generous funding from the NIHR Cambridge BRC. The funds were used to fully refurbish a large laboratory and purchase novel instrumentation enabling us to perform a wide range of immunoassays at unprecedented levels of sensitivity and reproducibility. Since its inception, the 7-strong CBAL team has issued over 1.2 million results. CBAL currently offers a range of over 500 different biochemistry tests. Many of these tests are novel immunoassays that were developed in-house.

CBAL provides an analytical service for BRC-funded researchers on the Cambridge Biomedical Campus and from other BRC sites around the UK. CBAL also provides support for researchers in many academic institutions around the UK and several small start-up commercial organisations. Most of the analytical work is performed on serum and urine samples.

Test prices are calculated to ensure that we recover costs for BRC projects and make a small profit from work performed for other organisations. This excess income is returned to the BRC.

Analytical work performed by CBAL has been presented in hundreds of scientific papers published in high-ranking journals. Topics cover many of the key Cambridge BRC themes.

CBAL offers efficient and cost effective sample analysis with a strong focus on quality assurance. A recent on-line poll of CBAL service users gave the laboratory a 98% approval rating. Many users of the CBAL analytical services have done so for more than 10 years.

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Introduction : What is CBAL ?

The Core Biochemical Assay Laboratory is funded by the NIHR Cambridge Biomedical Research Centre. Its primary function is to provide researchers access to Clinical Biochemistry assays that are not readily available from the local NHS Laboratory.

CBAL staff has a combined total of over 150 years service in the NHS. That experience is used to ensure that work performed by CBAL is of the highest quality.

CBAL Mission statement

1. To provide a high-quality sample analysis service.
2. To develop and optimise novel immunoassays as required.
3. To provide a timely and cost-effective service.
4. To respond promptly to any Project Requests or assay queries.
5. To ensure all work in CBAL conforms to local health & safety regulations.

Location & Postal Address

CBAL is located on Level 4 of the Laboratory Block at Addenbrooke's Hospital. The laboratory is situated in the IMS Annexe corridor.

Visitors can phone **01223 216728** to request entry or ask for help from the NHS

Pathology reception staff.

A detailed map of the Addenbrooke's Site is available on request.

The preferred address for packages delivered to CBAL is:

CBAL, Level 4, Laboratory Block

Addenbrooke's Hospital

Hills Road, Cambridge

CB2 0QQ

An Overview of the Core Biochemical Assay Laboratory (CBAL)

The Addenbrooke's NHS Clinical Biochemistry R&D Laboratory was established in the 1980's by the late Professor Charles "Nick" Hales to provide specialist assay support for his research interests, specifically the aetiology of non-insulin-dependent diabetes. The focus of the research was on the "thrifty phenotype hypothesis". He proposed that poor early (foetal and possibly infant) growth was associated with permanent changes in the structure and function of organs which predispose the adult to the development of non-insulin-dependent diabetes and the insulin resistance syndrome particularly in conjunction with adult obesity. Publications arising from this research led to interest from other research groups. The R&D Laboratory offered its analytical services to these groups as well. These unique services included not only insulin and c-peptide assays, but also assays for insulin precursors which were created using monoclonal antibodies which were generated in-house, or gifted from commercial collaborators. These assays for intact proinsulin and 32-33 split-proinsulin were adapted for use on a high throughput automated platform, making them suitable for large epidemiological studies. The assays are still in use today.

In 2008 funding from the newly-formed Cambridge Biomedical Research Centre was used to upgrade the laboratory facilities. The R&D Laboratory was renamed the Core Biochemical Assay Laboratory (CBAL) under the leadership of Keith Burling (Chief Biomedical Scientist and later Director of CBAL). In addition to meeting local, national, and sometimes international clinical needs, CBAL was now in a position to provide research assay support for both academic and commercial institutions.

Typically CBAL performs over 100,000 analyses each year. Professor Sir Steve O'Rahilly is the current Head of Department. His clinical interests are focused on obesity and insulin resistance. The list of publications associated with work conducted by the laboratory in these fields runs into the hundreds. Perhaps the easiest way to identify the academic studies CBAL has been involved with over the last 25 years is to look through publication lists from Professor Hales and Professor O'Rahilly.

Building on the laboratory's strength of development and evaluation of immunoassay, the current CBAL test repertoire provides both clinical and research collaborators with high throughput, cost effective, specialist assays. These include the adipose hormones leptin and

adiponectin; and PARC (Pulmonary and Activation Related Chemokine) as well as many cytokine and soluble CD biomarkers.

Leptin & Adiponectin were measured on the Ely & Fenland study cohorts in collaboration with Professor Nick Wareham, Head of the Cambridge MRC Epidemiology department. These assays are now are an essential part of the clinical investigation of obesity and insulin resistance. The PARC assay was developed in collaboration with Professor Tim Cox and Dr. Patrick Deegan in the Department of Medicine and is used to monitor the response to enzyme therapy in patients with Gaucher Disease.

CBAL also performed much of the early evaluation work on assays for the Bladder Cancer Biomarkers MCM5 & NMP22. The MCM5/NMP22 study was a collaboration between Professor Gareth Williams at UCL and Professor John Kelly at Addenbrooke's.

CBAL has a long record of successfully developing assays for start-up companies. One example is ProteinLogic for whom we measured a series of soluble CD markers in serum in an attempt to discover a 'fingerprint' of abnormal biomarkers in various disease states. This work has continued for almost 20 years. The current disease focus is Tuberculosis. After analysis of thousands of samples from sites around the globe ProteinLogic have identified a panel of useful biomarkers. ProteinLogic are now looking for partners to commercialise their research findings.

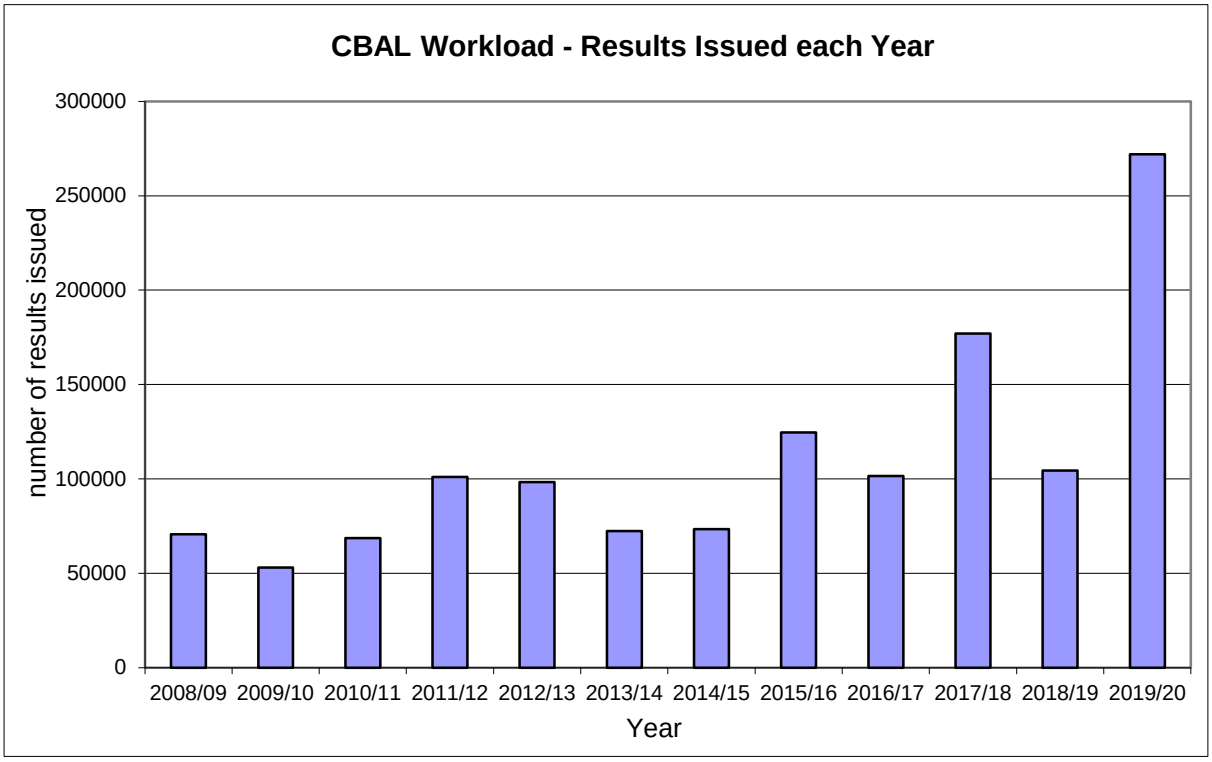
In 2007 CBAL was involved in a major study into predictive serum biomarkers for the early identification of pre-eclampsia. The study was a collaboration with PerkinElmer and Professor Kypros Nicolaides at Kings College, London. After measuring 15 biomarkers on over a thousand samples two were identified as good indicators of pre-eclampsia. These biomarkers are now becoming accepted as 'routine' clinical tests for this condition.

More recently CBAL has been involved with the evaluation of the Prostate Health Index (PHI) assay from Beckman Coulter. Results from these studies indicate novel uses of the assay which may be able to prevent unnecessary treatments for men with prostate cancer. CBAL has also been involved with INNODIA, a wide-reaching European Type I Diabetes Study. CBAL provides c-peptide measurements in both plasma and dried blood spot samples. The novel blood spot c-peptide assay took over two years to optimise satisfactorily.

In 2019 CBAL completed the largest study it had ever undertaken. It consisted of measurement of 54 cytokine biomarkers on near 3000 serum samples using MesoScale Discovery multiplex technology. The 54 assays were distributed over 7 different multiplex plates and generated almost 160,000 individual results. The whole project took nearly four months to complete and demonstrated the high level of teamwork within CBAL.

Workload

Since it's inception in 2008 CBAL has expanded the range of assays it offers and the number of tests it performs. The graph below shows the total number of results reported each year. The variability is caused by the inclusion of very large projects in some years. It is almost impossible to predict workload trends for the next five years but the likelihood is that demand will continue to increase.



Staff & Management Groups

CBAL currently has 7 permanent members of staff. Three are part-time.

The staffing structure currently includes a Laboratory Director, a Senior Biomedical Scientist, Four Biomedical Scientists and a Medical Technical Officer.

CBAL has a management committee which currently consists of Professor Sir Steve O'Rahilly, Professor Fiona Gribble, Dr Richard Kay & Dr Albert Koulman along with the CBAL Director. The committee meets 3 – 4 times each year to discuss laboratory performance and operational issues.

CBAL also has a 'virtual' Scientific Advisory Board. This is a group of highly respected scientists who will be asked for help & advice as required.

CBAL also has representatives on the Cambridge University Institute of Metabolic Science Health & Safety Committee and the IMS Annexe Management & General Purposes committee.

The CBAL Team *circa 2018*

Visitors

We will meet visitors by prior arrangement only.

To fit with our core working hour's meetings should be scheduled between 8am and 3pm.

Analytical Equipment Available In CBAL

CBAL has a range of automated and semi-automated equipment which enables us to process samples efficiently using high quality methodology. Several of the instruments are verified for use in human clinical diagnostic work.

DiaSorin Liaison XL

This is a fully automated analyser from DiaSorin in Italy.

https://www.diasorin.com/sites/default/files/allegati/ese_nuova_brochure_xl_per_web.pdf

The manufacturer has a wide range of assays available for this instrument.

https://www.diasorin.com/en/immunodiagnostic-solutions/clinical-areas

All methods are based on chemiluminescent immunoassay. There are many commonly requested human diagnostic assays covering thyroid, fertility, tumour marker and infectious diseases along with some more esoteric assays. DiaSorin are frequently expanding the assay range. It is not possible to transfer in-house assays onto this analyser.

Features of the instrument include stored calibration, bar-coded reagents and samples, minimal operator input, on-board QC monitor, on-board error detection. Analysis rates vary from method to method but may be up to 170 samples per hour. Results may be exported via an Excel spreadsheet.

CBAL primarily uses this instrument for c-peptide & insulin measurement but it has also been used for big studies measuring TSH, free T3, free T4, ferritin and others.

The performance of the instrument is excellent and during the 7 years it has been in the laboratory we have had very few breakdowns. Any issues have been dealt with rapidly under our comprehensive Service Contract.

Siemens Dimension EXL

This is a fully automated instrument from Siemens Healthineers. The analyser can measure a wide range of biomarkers using multiple technologies. Enzymes, Lipids, Cardiac markers and a range of commonly requested biochemistry tests can be measured using colourimetric chemistry. Sodium, potassium and chloride can be measured simultaneously using an integrated electrode. A further selection of biomarkers can be measured by three forms of immunoassay (turbidimetry, LOCI & magnetic particle).

https://static.healthcare.siemens.com/siemens_hwem-hwem_ssxa_websites-context-root/wcm/idc/groups/public/@global/@lab/@corelab/documents/download/mda5/ntg3/~edisp/30_19_14118_01_76_dimension_exl_menu_oct_2019_final-07066839.pdf

Features of the instrument include stored calibration, bar-coded reagents and samples, minimal operator input, on-board QC monitor, on-board error detection & automatic dilution of samples with concentrations above the assay range.

Analysis rates vary from method to method but may be over 100 samples per hour. Assays use very low sample volumes and 'dead volume' sample requirements are very small – thus making this instrument ideal for processing a lot of assays on minimal sample volume. Results may be exported via an Excel spreadsheet. The instrument is capable of analysing a

range of primary & secondary sample tubes with and without barcodes. Small samples can be analysed in micro-sample cups.

CBAL primarily uses this instrument for the measurement of glucose, cholesterol, HDL, triglycerides, urea and creatinine. Many other methods have been tried. Although most work is done with Siemens reagents the instrument has been successfully used to run in-house colourimetric assays such as bile acids, fructosamine & glycomark using reagents from other vendors.

We have had a Siemens Dimension in the laboratory for over 15 years. The combination of high throughput, low sample volume and reliability make it an essential instrument.

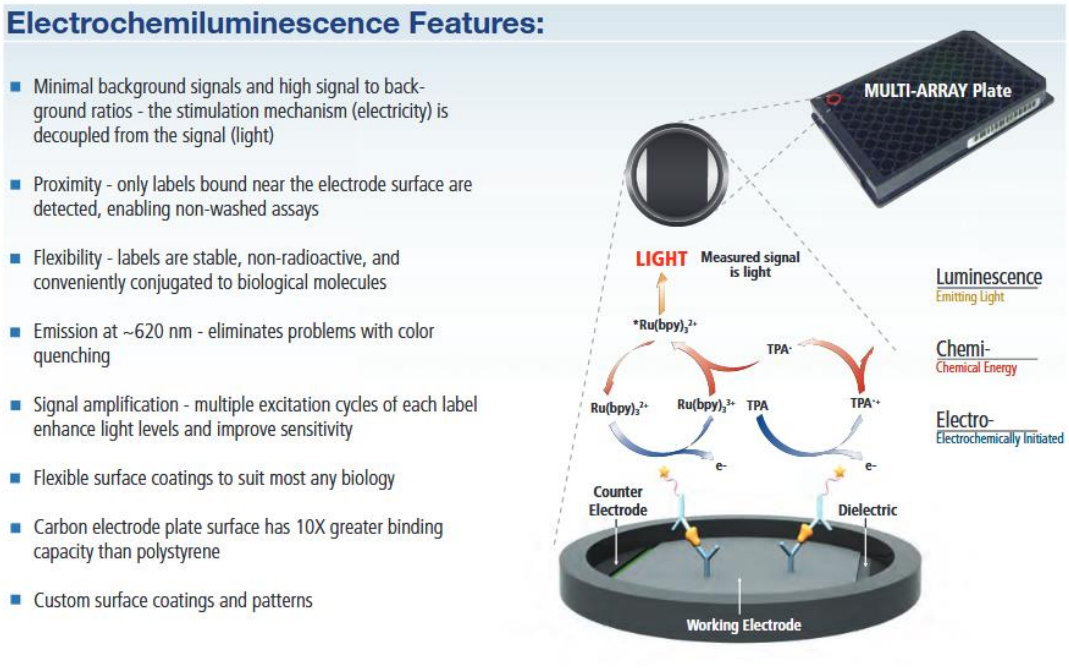
MesoScale Discovery Sector s600

This instrument is a plate-reader for MesoScale Discovery assays.

MSD are based in the USA. All kits and reagents are sent from the USA. Their analytical methods and assay validation details are presented in great detail on their website (www.mesoscale.com).

MSD assays are based on electrochemiluminescence immunoassay. Assays are performed in 96-well black microtitre plates with electrodes incorporated into the base. Most assays employ a standard 2-site immunoassay protocol and take between 3 and 5 hours to complete. We can comfortably run up to 8 MSD plates in a day. Analysis of samples in duplicate typically takes between 25 and 50µl of sample.

In MSD assays the capture antibody is immobilised as a ‘spot’ on the base of the plate. The detection antibody has a luminescent label. When the assay is complete a read buffer is added to each well and the plate is loaded into the reader. A high voltage is applied to the bottom of the plate which causes the label to emit light. The amount of light released is determined by a high-power CCD camera.



The instrument converts the light emitted into a numerical value. These values are transferred to the MSD Workbench software package which generates calibration curves and calculates the concentration of unknown samples. Reading a full 96-well plate takes less than 2 minutes. All data is stored on the MSD computer and a data summary is taken for each assay and stored on the CUH Trust server.

MSD are constantly revising their product range. We have used their assays for over 10 years and find their products to be some of the best-performing and cost-effective available. MSD kits have excellent batch-to-batch reproducibility, impressive lower limits of detection and a very wide working range.

Here is a summary of the different assay types that are currently available.

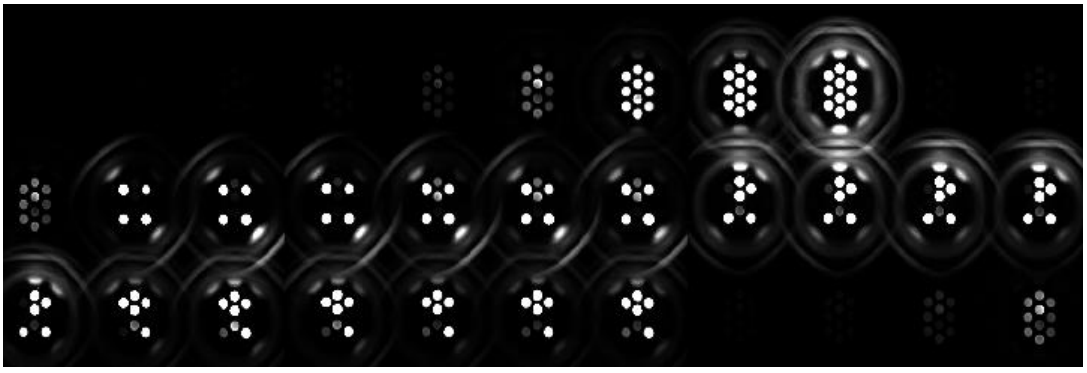
V-Plex

This is our preferred assay type because the V-plex range of assays has the highest degree of validation and the best batch-to-batch reproducibility. There are a wide range of assays available from MSD. Some are singleplex (one assay in each well), some of multiplex (up to 10 assays in each well). Multiplexing enables up to 10 different biomarkers to be measured simultaneously. Each biomarker has its own capture antibody spot at the base of the well. General rules of multiplexing are that the analytes in the panel must be in approximately the same concentration range and they must not be so similar that antibodies for a different biomarker cross-react. Multiplexing has some obvious advantages in terms of result throughput and sample volume requirements. However, we believe there is some loss of assay sensitivity and occasionally we see spot-to-spot cross-reactivity when samples have extremely high analyte concentrations (as seen with cytokines generated in cell cultures).

We have used most of the human V-plex panels sold by MSD.

https://www.mesoscale.com/en/products_and_services/assay_kits/v-plex/v-plex_assay_menu

A picture from a 10-plex assay is shown below. Each spot represents a different cytokine.



We have had considerable experience with the 10-plex Pro-Inflammatory Cytokine panel. One important point to note about the cytokine panels is that even with high-sensitivity MSD assays it may not be possible to measure all of the cytokines in 'normal' human serum.

U-Plex

U-plex assays are relatively new. These assays offer the opportunity to create a pick-and-mix multiplex panel. There are currently around 80 assays to choose from. MSD's online panel designer ensures incompatible assay combinations are not selected.

https://www.mesoscale.com/en/products_and_services/assay_kits/u-plex_gateway/u_plex_designer

U-plex assays require the user to perform their own capture antibody coating using special microplates and 'linkers'. The assays are validated to a high standard but they are unlikely to have the same long-term reproducibility as the V-plex range.

S-Plex

The S-plex range of assays was released in 2019.

https://www.mesoscale.com/en/products_and_services/assay_kits/s-plex

The product range is currently quite limited. It focuses on low-concentration biomarkers which have proved difficult to measure using conventional MSD assays. S-plex assays employ slight modifications to the traditional MSD assay format. Sensitivity is said to be at least 10 times better than V-plex assays.

The research value of S-plex assays is still uncertain. There is very little evidence to show that the improved sensitivity actually adds value to research studies. The assays are considerably more expensive than conventional MSD kits.

R-Plex

R-plex assays are sold as 'assay development' kits. All components (antibodies, calibrator & assay diluents) are included. Plates are purchased separately. MSD supply an assay protocol and a small amount of validation data.

There is a wide and constantly expanding range of assays available in R-plex.

https://www.mesoscale.com/en/products_and_services/assay_kits/r-plex

These assays tend to be for newly emerging biomarkers and those in low demand. If the assays become popular it is possible they will be converted into the U-plex or V-plex formats. We have found R-plex assays to be a better option than ELISA.

In-House MSD Assays

Use of MSD's 'standard bind' blank plates enables us to develop our own assays on the MSD platform. This is our method of choice for assay development.

The blank plates allow capture antibody to bind across the carbon base of the wells rather than in a spot. We tend to select a biotinylated detection antibody and use the addition of Streptavidin SulphoTAG from MSD to generate the luminescence.

We have created over 100 assays in this way. Most of these have been of a very good quality. Successful assay development is very dependant on the quality of antibodies we are able to use. DuoSets from BioTechne are ideal for this purpose.

Developing assays in MSD format can sometimes lead to a 100-fold improvement in sensitivity over the same reagents used in conventional ELISA. In addition, we usually see a considerable improvement in assay range and a reduction in the sample volume required. Our starting sample volume is 10µl per well – occasionally it needs to be increased. MSD assays usually require less antibody than ELISA but this is offset by the higher cost of the blank plate.

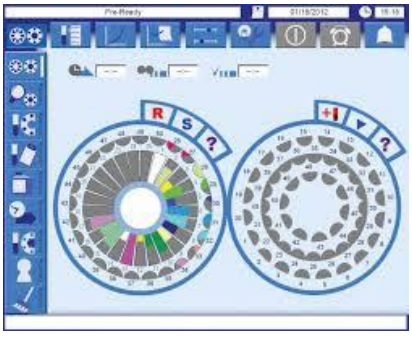
The most frequently performed in-house MSD assay is GDF-15. This has been used in multiple research studies over the past 5 years. We are also currently involved in two very large studies looking at C-Peptide concentrations in Dried Blood Spots. Optimisation of this assay took over two years and required careful selection of antibodies, assay diluents and extraction protocols. The in-house assay proved to be more sensitive than any of the commercially available assays.

Randox Daytona +

This is a fully automated analyser from Randox in County Antrim, Northern Ireland.

The manufacturer has a wide range of assays available for this instrument. Methods are based on colourimetric chemistry or turbidimetric immunoassay. Many of the assays are identical to those available on the Siemens Dimension. The Daytona+ was purchased with the intention of running specialist assays that were only available through Randox (such as Bile acids, Lp(a) and the apolipoproteins).

https://www.randox.com/rx-daytona-plus-overview/



As with the Siemens Dimension, features of the instrument include stored calibration, bar-coded reagents and samples, minimal operator input, on-board QC monitor, on-board error detection & automatic dilution of samples with concentrations above the assay range. Many of the functions can be accessed through the user-friendly software (above).

One major problem we have discovered is the inability of the instrument to work with sample volumes much smaller than 500µl. This is much greater than the minimum sample volume quoted by Randox. This high sample requirement represents a real challenge for many of the projects we undertake.

BioRad Luminex Magpix

The Magpix is a multiplexing immunoassay platform which utilises antibody-coated paramagnetic beads that are internally dyed with red and infrared fluorophores of differing intensities. Each dyed bead is assigned a unique number, allowing the differentiation of one bead from another. Multiple analyte-specific beads can then be combined in a single well of a 96-well microplate-format assay to detect and quantify multiple targets simultaneously.

Magpix assays can be highly multiplexed. It is possible to run up to 50 assays simultaneously.

The Magpix assay uses conventional 2-site immunoassay protocols in which the capture antibody is immobilised on a microbead rather than the walls of a microtitre plate. The assay is performed in a microtitre plate and wash steps are required. These are performed using a special automated plate washer which uses a strong magnetic field to trap the beads at the bottom of the assay plate while washing takes place. For most assays the antibodies are biotinylated and the fluorescent signal reagent (phycoerythrin) is coupled to Streptavidin. Plate reading is done on the Magpix instrument. It typically takes 60 minutes to read a 96-well plate.

Calibration curve generation and result calculation is done using the BioRad BioPlex Manager software package. Results may be exported in Excel spreadsheet format.

CBAL has only used commercially-available kits on the Magpix. It is possible to set up in-house assays on this platform but MSD is used in preference. There are at least three manufacturers of Luminex kits. Luminex have created an assay finder website which is a good place to start a search for assays.

https://kitfinder.luminexcorp.com/

Further searches can be performed on BioTechne, ThermoFisher & Millipore websites.

The Magpix technology gives the opportunity to measure a very high number of analytes simultaneously. High-plex kits do become very expensive (well over £2000 each) so it is essential to plan experiments carefully. Smaller plexes can also be run with a limited ability to pick-and-mix assays. The range of Magpix assays available is greater than other platforms. However, the sensitivity and overall performance of Magpix assays appears to be inferior to MSD.

PerkinElmer AutoDELFIA

DELFIA (Dissociation Enhanced Lanthanide FluoroImmunoAssay) may be regarded as one of the first high-sensitivity Immunoassay techniques. DELFIA measurements are based on Time-Resolved Flurorescence. DELFIA methods are generally singleplex and may be competitive or non-competitive.

The AutoDELFIA is a batch analyser and not really suited to modern clinical laboratory requirements of rapid throughput of multiple analytes. This is unfortunate as DELFIA assay kits were amongst the best available in terms of accuracy and reproducibility. Due to decreased demand kits for the measurement of TSH, free & total PSA, insulin, c-peptide and many others have now been withdrawn from sale. There are now very few commercial kits apart from those used for neonatal and ante-natal screening.

The AutoDELFIA is sold as a closed system – preventing users from transferring their own methods to the platform. Fortunately, staff in CBAL have a good working relationship with PerkinElmer and have been granted access and training on the AutoDELFIA software. Much of the early insulin precursor work was done on the AutoDELFIA. Now only our in-house leptin, adiponectin and intact proinsulin assays are performed on the analyser.

The AutoDELFIA automates the whole of the immunoassay process. DELFIA assays are run on antibody-coated microtitre plates. These are loaded onto the instrument along with samples, assay diluents and wash buffers. The tests required are requested via the instrument software. When loading is complete the instrument works through the assays required. Capacity is limited but the AutoDELFIA can run up to 8 different assays at a time. Samples can be removed from the instrument after plate loading is complete. The remainder of the process is truly walk-away. The AutoDELFIA can be left to complete assays overnight if required.

Tecan EVO-100 Automated Liquid Handling Robot

The Tecan Evo-100 is used for studies where high-throughput is required. For example we used it for a project where 10 separate microtitre-plate assays were performed on a set of 35 samples. The Tecan handled sample distribution easily with minimal operator intervention. We use the Tecan for plate loading only. We do not have additional modules which would enable us to complete the immunoassay. The Tecan is also invaluable for handling samples regarded as ‘high-risk’ , the only user intervention is loading samples into

the Tecan sample racks and unloading them when all pipetting steps are complete. When all work has been completed the instrument may be decontaminated easily.

PerkinElmer Dried Blood Spot Puncher

This device is essential for the analysis of dried blood spot samples. It is primarily used for the C-Peptide assay but other assays have also been set up.

Our 'standard' blood spot size is 3.2mm but larger or smaller sizes can be created by changing the punch head. Multiple punched spots can be taken from a single blood spot.

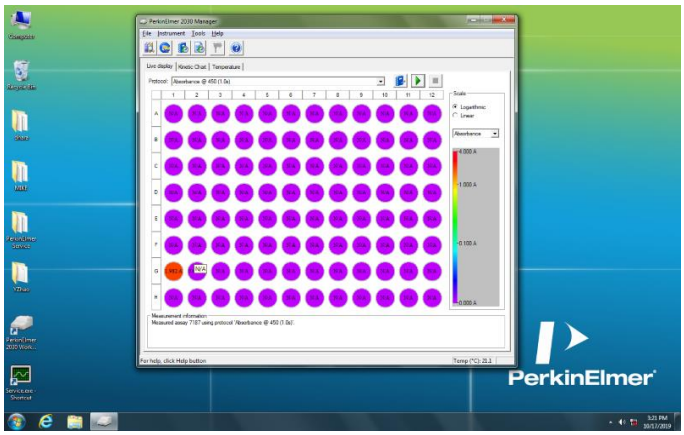
Our experiences with DBS collection show that poorly-spotted sample cards are likely to be a major contribution to assay inaccuracy.

PerkinElmer Victor-3 Plate Reader

The Victor-3 Plate reader is used for the analysis of 96-well microtitre plates. Plates are analysed in 3 modes, absorbance, time-resolved fluorescence & luminescence.

Absorbance is primarily used for end-point reading of an ELISA assay. The wavelength most commonly used is 450 nm (the colour produced in assays with TMB substrate). Other wavelengths are available. Background correction can be applied if required. Time resolved fluorescence is used as the end-point of DELFIA assays. Luminescence readings are limited to assays producing a long-lasting luminescence product. Our set-up is not suitable for shoot-and-read luminescence assays.

The instrument software is user-friendly and can be easily modified to read most plate formats and assay set-up's.



Data from the plate readings may be fed into the onboard software package (MultiCalc) to generate standard curves and calculate results. .Although this software is over 30 years old it still has functions that surpass those from contemporary programs. Data can be exported from Multicalc in a format that can easily be imported into Microsoft Excel.

ELISA (Enzyme-Linked ImmunoSorbent Assay)

ELISA is almost certainly the most commonly performed immunoassay technique in the world today. Unfortunately, there is a common perception that immunoassays like these are easy to perform and infallible. Our experience shows the opposite. Poorly performed ELISA experiments using inferior assay kits can yield very misleading results.

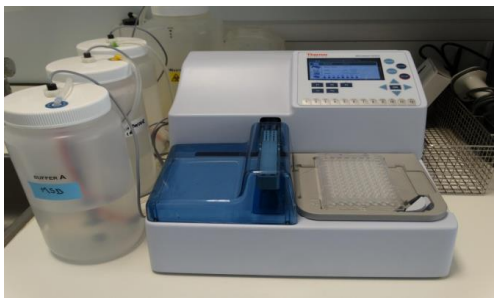
Our very experienced CBAL staff can help with the selection of appropriate high-quality assays and advise against the use of poorly designed kits. In general, we look for kits where the manufacturers show a significant amount of assay performance data along with simple and well-designed assay protocols. An excellent example of such a company is R&D Systems

(BioTechne). Kit handbooks for their Quantikine range of assays are excellent and show most of the information required.

There are many manufacturers & distributors of poor ELISA kits. Some assays are sold through several different manufacturers with minimal changes of packaging. These kits do not work well and have very little supporting documentation about the performance of the assay. Some of the worst kits are those that give no information about appropriate sample dilutions for the sample matrix under test (serum, urine etc..). This implies samples have not been tested. In addition, these kits are often extremely expensive (over £500 for a 96-well plate). We will usually advise against use of these kits unless it is totally unavoidable.

Other Equipment

CBAL has three plate washers and numerous plate shakers (one temperature-controlled) which enable us to maintain our high-quality, high-throughput assays.



Instrument Maintenance & Service Contracts.

CBAL staff ensures that all of the instrument manufacturer's recommended Daily, Weekly & Monthly maintenance procedures are completed and recorded on our database.

All of the major pieces of equipment in CBAL are covered by Manufacturer's service contracts. These contracts include a Preventative Maintenance service visit at least once a

year and priority status should an instrument breakdown occur. The cost of service contracts for all of CBAL's instruments exceeds £70,000.

Using equipment – Other departments

Very occasionally we will be able to let researchers from other groups use our equipment. This offer is most likely to relate to the MesoScale or Luminex plate readers.

This facility can only be offered if the researcher contacts CBAL well in advance and they follow CBAL safety guidelines and other rules. A member of CBAL staff must be available to supervise the work. Therefore, instrument sharing can only take place during the standard working day (no weekend or evening work). A small charge may be made for this service. It is often more beneficial for the whole process to be undertaken by the CBAL team.

Other Assays

Vitamin C

We have used an in-house fluorescence assay for Vitamin C for almost 20 years. The assay is reliable but correct sample collection protocols are critical for accurate results. Recently, we have been able to analyse samples from an EQAS scheme (see page 49). It was reassuring to see that CBAL results fell at the centre of the all-laboratory distribution.

NEFA (Non-esterified free fatty acids)

CBAL uses the colourimetric Free Fatty Acid microtest kit from Roche. The assay protocol has been modified to enable us to run the assay in microtitre plate format. This reduces the amount of sample required and allows us to run large numbers of samples efficiently.

3OH-Butyrate

CBAL uses the colourimetric 3-OH butyrate kit from Stanbio (via Aspect Scientific). The assay protocol has been modified to enable us to run the assay in microtitre plate format.

Glycerol

CBAL uses the colourimetric Glycerol Assay kit from Sigma.

Human Tissue Act (HTA) & Sample Receipt & Sample Storage

CBAL is covered by the Cambridge University Hospital's HTA Licence.

Some areas within the IMS Annexe Corridor (which is predominantly occupied by University of Cambridge research groups) have been clearly marked as belonging to CBAL & the NHS. These areas may contain human serum or urine samples with various forms of labelling. Freezers containing CBAL samples are also clearly marked for NHS use only.

Sample Receipt

It is extremely important that the specimens sent to CBAL are labelled with sufficient clarity to enable us to match them to the work list we receive from you. We advise using simple unique sample identifiers so they are easily recognised.

Please use a permanent fine tip marker if you are writing directly onto the tubes. Most tubes have a rough area that is specific for this use. Be aware that even permanent ink has the potential to rub off smooth surfaces during defrosting. Mislabelled or poorly labelled samples will not be analysed.

Printed labels are welcome but they have a tendency to fall off at temperatures below -20° degrees C. For larger studies only, CBAL offers a barcode printing service. These labels are suitable for use with Sarstedt 2.0mL type tubes only. Email CBAL to ask about this service. There will be a small charge for the labels. Please note we cannot prepare labels 'urgently'.

Examples of acceptable tube types:

- Sarstedt 2.0mL microtubes
- (product code 72.694.406) micro-centrifuge tubes of various sizes (0.5 – 2.0mL)
- Cryovials (sigma / thermo)
- Eppendorf safelock

We have modified our sampling protocols to enable us to process samples sent in 96-well barcoded cap-cluster tubes (shown below). However, please avoid dispensing your samples directly into standard microtitre plates as they are very difficult for us to sample from, easy to spill and can lead to mistakes in sample identification.

Please follow these guidelines when sending samples to CBAL.

The documents in Appendix 2 & 3 of this brochure will provide useful information.

Put the samples in a box or bag that is adequately labelled with the following details :

- Researcher name
- Date of delivery
- Number of samples
- Analysis required

The picture above shows 3 ways we prefer to receive samples from researchers. The samples should be accompanied by a hard copy worklist of the specimens sent.

Specimens should be brought to the laboratory in a box, preferably transported on dry ice, according to **local regulations**. Ensure the box will not open, even if turned upside-down.

When a batch of samples is received in CBAL we will give it a laboratory batch number and book the request into our sample receipt database. The database contains details of where samples are stored in the laboratory freezers. The database is updated as results become available and when the results are reported back to the researcher. All freezers are covered by a temperature monitor system. A visual signal alerts staff to any potential problems.

We have limited sample storage facilities. We can keep samples for a maximum of 3 months after the work has been completed. If samples are no longer required please let us know via email. The samples will be discarded following local rules. If you need to keep the samples please come and collect them. It would be wise to let CBAL know that you are coming. If we have not heard from you within three months the samples will be discarded.

Worklists

We will ask you to send an Excel spreadsheet to accompany samples submitted to the laboratory. The spreadsheet should contain a list of sample IDs and the tests required. It is essential that we receive an electronic copy (sent to cbal@addenbrookes.nhs.uk) but an additional paper copy may also be useful. We will use the spreadsheet to create worklists for the analysis. Please do not send PDFs.

For confidentiality reasons we would prefer to receive spreadsheets with sample IDs on them rather than full names. If sending patient-identifiable information it is essential that the spreadsheet is sent via a secure server (nhs.net to nhs.net). Please contact CBAL for further details. Other email options are unacceptable.

When delivering samples it is vital that we receive a list of tests that are required on each sample. Failure to send a spreadsheet & test list will lead to delays in the processing of your samples. If we have to create a spreadsheet ourselves there may be an additional charge.

Sample Collection & Labelling

We will give advice on sample collection wherever possible. The advice we give will be based on information from kit manufacturers and text books and from our experiences with previous studies. Some assays will require specific anticoagulants and/or pre-treatment at the time of collection. Therefore, it is always advisable to discuss sample requirements for assays whilst planning the study or at least prior to commencing sample collection

We are frequently asked about stability of biomarkers in various sample types. We often have some in-house data but this will be on a limited number of samples and over a short time frame. Analyte stability experiments are occasionally reported in the literature. However, true long term analyte stability data is almost impossible to acquire.

Serum

We can analyse serum prepared by traditional clotting methods or from tubes containing a clot enhancer and gel to aid separation when the sample is centrifuged. We have no evidence to show that these collection methods give different results but there are a few publications stating that they do.

Plasma

We will attempt to analyse samples collected with a range of anticoagulants. However, in our experience, the two most useful are EDTA or lithium heparin. Some plasma preparation tubes have specific inhibitors to preserve the analyte under test (for example fluoride oxalate – for glucose & lactate). Some anticoagulants, such as EDTA, work by inactivating essential clotting factors within the samples by removing divalent metal ions. Therefore, EDTA samples will be unsuitable for measurement of calcium, magnesium and enzymes reliant upon the co-factors such as alkaline phosphatase. Citrate plasma should be avoided.

General Precautions for preparation of serum & plasma.

It essential blood samples are centrifuged promptly and the serum or plasma transferred to an aliquot tube and frozen. Failure to do this will lead to unreliable results. Care must be taken to avoid sample haemolysis during venepuncture. Slight haemolysis usually has little effect on results but gross haemolysis (deep red plasma or serum) may invalidate analysis of the sample completely. Method-specific issues will be discussed in the 'tests available' section.

Comparison of results from paired serum & plasma samples.

We have a limited number of commercially-sourced paired serum/lithium heparin plasma/EDTA plasma samples which are used to evaluate methods. The one drawback of these samples is that they are from 'normal' subjects so concentrations of some biomarkers are extremely low. It is very difficult to get paired samples from individuals with a range of diseases.

Platelet Free Plasma

Some biomarkers (usually those involved in the blood clotting process) can have very different concentrations in serum and plasma. We will pass this information on to the researcher if we have it. Examples of these biomarkers are BDNF, AGRP, MMP-9 & TIMP-1.

In circumstances such as these the advice from manufacturers such as R&D Systems is to use platelet-free plasma. This plasma is obtained by collection of whole blood into EDTA or lithium heparin anticoagulant. The blood is then centrifuged promptly and plasma removed as usual. The plasma is then centrifuged again at higher speed (10,000 rpm for 5 minutes). The upper 90% of the plasma is then carefully transferred to a second tube. The lower layer (containing platelets is discarded).

Whole Blood

Occasionally, we will perform tests on whole blood. By far the most common of these is HbA1c. A fresh or recently-frozen whole blood sample is essential for reliable results.

Urine

CBAL offers a range of assays on human urine. Unless informed otherwise, we required urine without preservatives. The volume required is usually quite low. This will be discussed at initial enquiry. Often we will recommend measurement of urine creatinine as well to act as a normalisation factor. Results will be reported as an analyte/creatinine ratio.

Cell Culture

Many of the assays performed by CBAL can be adapted to cover the range of concentrations found in cell culture samples. Often a simple change in sample dilution will bring the results into range. Extremely high concentrations of cytokines found in cell cultures may interfere with the assay technology. In the MSD 10-plex proinflammatory cytokine multiplex we have encountered samples where massive concentrations of 3 of the cytokines have interfered with results from other low-concentration cytokines in the multiplex. It is almost impossible to eliminate this interference without running samples at multiple dilutions – this has a significant cost impact.

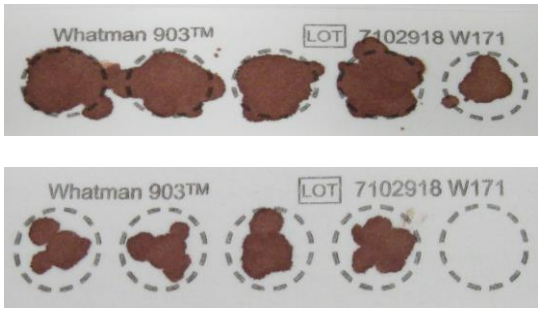
CSF

CBAL can offer a range of assays on CSF samples. Occasionally, assay protocols will need to be modified to cover the different analyte concentrations found in this fluid. It is essential to avoid blood contamination to obtain meaningful results.

Dried Blood Spots

CBAL has considerable experience with the analysis of dried blood spots. Although analysis of this sample type has obvious advantages for the subject, particularly when collecting samples at multiple time points, it should be noted that poor result reproducibility may be a significant issue. There are two main causes of unreliability. Firstly, the quantity and uniformity of the blood spots submitted to us. This is particularly apparent when samples are collected by the subjects themselves. In our experience even highly trained nursing staff may occasionally submit samples that are unsuitable for analysis.

Examples of acceptable (top) and poorly-collected (bottom) samples from patients are shown below



The second cause of poor results is related to the actual amount of serum that is released from a spot during elution. On average, a standard 3.2 mm spot punched from a 'guthrie-card' collection device yields just under 2µl of plasma. Therefore using 4 spots we can only obtain a maximum of 8µl plasma – which is far less than the recommended 25µl of plasma usually required for the assay. These volumes assume 100% recovery when eluting the spots which is unlikely to occur.

Many DBS cards we receive have been in long-term cryostorage. Unfortunately, we are not able to offer much advice regarding the stability of biomarkers in the DBS during that time.

Biochemical Tests available from CBAL

CBAL is pleased to offer a range of over 200+ ready-to-use assays. We will have reagents for many of the tests in-stock but others will need to be ordered as required.

The CBAL repertoire has some overlap with the local NHS Blood Sciences laboratory. If we receive a project request where none of the assays are exclusive to CBAL we will pass the request on to the Laboratory Research Services team (LRS@addenbrookes.nhs.uk). Similarly if LRS have requests that are more suitable for CBAL they will pass them on to us.

Below are tables with list of tests available for each of the instruments in CBAL. The list gives an indication of the range of tests available. If you require a test that does not appear on the list please contact CBAL and we will try to source it for you.

It is important to note that we can supply virtually any commercial test produced for the DiaSorin Liaision XL, Siemens Dimension, Luminex Maxpix, MesoScale Discovery s600 & Randox Daytona+.

Examples of the Assays on the Siemens Dimension EXL

Albumin	Aldolase	Alkaline Phosphatase	ALT
Amylase	AST	Bilirubin	Caeruloplasmin
Calcium	Cholesterol	CK	CK-MB
Creatinine	CRP (regular & hs)	Electrolytes (Na/K/Cl)	Folate
Gamma GT	Glucose	HbA1c	HDL
IgG	IgA	IgM	Inorganic Phosphate
Iron	Lactate	LDH	Lipase
Magnesium	NT-proBNP	TIBC	Total Protein
Transferrin	Triglycerides	Troponin I	Urea
Uric Acid	Urine Albumin	Urine Total Protein	Vitamin B12

EDTA plasma is unsuitable for calcium, magnesium, alkaline phosphatase, electrolytes and others.

HbA1c – the most accurate and reliable results are obtained on fresh whole blood with EDTA anticoagulant. Please note that the Laboratory Research Services team should be the first contact for HbA1c measurement.

Haemolysis will affect many assays including folate, glucose, inorganic phosphate, magnesium, iron, TIBC. Gross haemolysis will invalidate most biochemical measurements.

LDL may be calculated using the Friedwald formula. Direct LDL measurements can be performed on the Dimension after prior consultation with the laboratory.

The 'regular' CRP assay will be sufficient for most projects. The lower limit of detection is approximately 1 mg/L. The 'high sensitivity' version of the assay is primarily used as a cardiac risk marker. The lower limit of detection is approximately 0.2 mg/L. The high sensitivity assay is three times more expensive.

Grossly lipaemic samples present many difficulties when performing biochemical analysis. We aim to minimise the issues using a combination of high-speed centrifugation and sample dilution but some assay interference is still highly likely.

Fluoride Oxalate plasma is required for glucose measurements. Other types of plasma and serum will give valid glucose results provided the whole blood has been centrifuged promptly and the serum/plasma taken off the red cells and frozen.

Fluoride oxalate plasma is the only recommended sample type for lactate measurements. Whole blood samples should be kept on ice and centrifuged within 30 minutes of collection. The resulting plasma should be stored at -20 degrees C until analysis. Other sample types are unsuitable as lactate concentrations rise rapidly in them.

Bicarbonate (total CO_2) can be analysed on the Dimension. Correct sample collection is essential for accurate results. Incompletely filled whole blood or aliquot tubes will give a false result due to absorption of CO_2 from the air into the sample.

Examples of the Assays on the DiaSorin Liaison XL

1-25-OH Vitamin D	25-OH Vitamin D	Aldosterone	Androstenedione
CA 15-3	CA 19-9	CEA	Cortisol
C-Peptide	DHEAS	Estradiol	Ferritin
Free T3	Free T4	FSH	Growth Hormone
IGF-I	Insulin	LH	Prolactin
Progesterone	Renin	SHBG	Testosterone
Total T3	Total T4	TSH	Urine C-Peptide

Samples for insulin & c-peptide measurement should be centrifuged promptly and the serum/plasma frozen away until analysis. We have some evidence that indicates that sample storage should be at -70 degrees C as some degradation can occur within months at -20 degrees C.

Urinary c-peptide can only be offered as a research assay. Please contact the CBAL for advice about sample collection, preservation and storage. Clinical samples for Urine c-peptide are sent to an external laboratory.

Examples of the Assays on the MesoScale Discovery s600 (commercial kits)

Abeta 38-40-42	CXCL-10 (IP-10)	FLT-1	GIP
ICAM-1 (CD54)	IFN gamma	Il-1beta	IL-2
IL-4	IL-5	IL-6	IL-8
IL-10	IL-13	MCP-1	MCP-4
MDC	MIP-1alpha	MIP-1beta	Osteocalcin
Osteoprotegerin	Osteonectin	PLGF	PYY
Resistin	SAA (Amyloid A)	TARC	Total GLP-1
Total Active GLP-1	TNF alpha	VCAM-1 (CD106)	VEGF

The list above includes the most frequently measured analytes. The list of assays available grows regularly as do the variations of the MSD methodology. (V-plex, U-plex, S-plex, R-plex etc...)

Certain biomarkers (GLP-1, PYY & GIP) will require Trasylol/DPPIV inhibitors adding to the whole blood sample to act as preservative. The Beckton Dickinson P800 sample tube is a suitable alternative.

Some biomarkers may be present in different concentrations in serum and plasma. CBAL will pass this information on to the researcher if it is available.

In-House Assays on the MesoScale Discovery s600

Afamin	BDNF	CD14	CD25 (IL2R alpha)
CD26	CD27 (TNFRSF7)	CD32 b/c	CD33
CD35 (Comp Rec 1)	CD40 (TNFRSF-5)	CD40L (TNFSF5)	CD50 (ICAM-3)
CD56 (NCAM)	CD62-L (L-selectin)	CD85j (ILT-2)	CD87 (uPAR)
CD117	CD120b	CD121b (IL-1 rII)	CD137 (TNFRSF9)
CD150 (SLAM-F1)	CD152 (CTLA-4)	CD163	CD166 (ALCAM)
CD170	CD222	CD239 (BCAM)	CD263 (Trail R3)
CD282 (TLR-2)	CD324 (E-Cadherin)	CDw329 (Siglec 9)	Complement Factor H
CXCL-9	DLK-1	EGFR	GDF-15
Granzyme A & B	Lipopolysaccharide-BP	PAI-1 (Serpin E1)	PD-1
Pentraxin-2	PLAUR (uPA)	PSP-94	Serpin G1
Serpin A4 (Kallistatin)	SIK-1	TIM-3	TNFRSF25 (DR3)

All of these assays have been set up in-house using commercially available antibodies and calibration materials. The assays on the list have all undergone a validation process which ensures the results they generate are valid. Inevitably, some assays will perform better than others. We are happy to share validation data if requested.

We have at least 20 more assays that have undergone the set-up process and failed. They have failed either because the antibodies are incompatible or because they are unable to detect the biomarker of interest in the sample matrix (human serum/urine etc..).

Examples of the Assays on the PerkinElmer AutoDELFIA (or Manual DELFIA)

all except AFP are in-house methods

Adiponectin	AFP	High Sensitivity A1-AT	Intact ProInsulin
Leptin	PARC	Urine RBP	

Examples of the Assays on the Randox Daytona+

Apolipoprotein A-I	Apolipoprotein A-II	Apolipoprotein B	Apolipoprotein E
Bile Acids	Fructosamine	Glutamine	Glycated Albumin
Glycomark	Haptoglobin	Lipoprotein (a)	Orosomucoid

Examples of the Assays on the Luminex Magpix

MMP-1	MMP-3	MMP-7	MMP-8
MMP-9	MMP-10	MMP-12	TIMP-1

The assays listed above represent a fraction of those we can perform on the Magpix. As a general rule if an assay can be performed by either MSD or Magpix we would strongly recommend using the MSD assay as from our experience the assay performance is likely to be better.

Examples of the Assays performed manually using ELISA kits

Alpha Klotho	Angiopoietin-2	FGF-21	FGF-23
Ghrelin (total or active)	Glucagon	Hepcidin	Hyaluronic Acid
IGFBP-3	IsoInsulin	Lp-PLA2	Ox-LDL
Salivary Cortisol	Salivary DHEAS	VEGF-R2	ZnT8 Antibody

Samples for Glucagon analysis will require inhibitors adding to the whole blood sample to act as preservative. The Beckton Dickinson P800 sample tube is recommended.

Salivary kits are purchased from Salimetrics. The manufacturer has recommended sample collection procedures for each analyte. Please check with the laboratory before proceeding with a study. Collection devices can be purchased through Stratech who distribute Salimetrics products in the UK.

Most of the kits mentioned above all come from suppliers that we believe to be reliable, namely, Salimetrics, R&D Systems, Mercodia, & Millipore. The documentation supplied with these kits gives us some assurance that the product will work as advertised. We are very wary of manufacturers who supply products with little or no validation data.

Assays performed Manually

3-OH Butyrate (Alere)	Glycerol (Sigma)	NEFA (FFA) (Roche)	Vitamin C (In-house)

Haemolysis should be avoided for all of these assays. We have 'blank' correction procedures in place for the 3-OH butyrate and NEFA assays which ensures that moderate haemolysis has little effect.

We have some evidence that concentrations of 3OH-butyrate and NEFA are unaffected by sample type. We have short term stability data that suggests concentrations are unaffected by short term storage. We have no information on the effect of long-term sample storage on NEFA or 3-OH butyrate concentrations.

Sample collection for Vitamin C is critical. Lithium Heparin plasma is the only validated sample type. Lithium heparin blood tubes should be centrifuged promptly after collection and the plasma should then be deproteinised immediately with Metaphosphoric Acid and frozen. Please contact CBAL for further details.

Clinically Validated Assays

The CBAL laboratory has provided 'clinical' results for hospital patients for many years.

These samples are handled in a different way to research samples. CBAL has six 'clinical assays'. Two of these are covered by UKAS ISO 15189 accreditation. The others have high-quality long-term assay performance data but this would be considered insufficient to obtain and maintain UKAS accreditation.

The two assays within scope of UKAS accreditation are insulin & c-peptide. These assays are run on the DiaSorin Liaison XL autoanalyser. Please note that we do not offer urinary C-peptide analysis on clinical samples.

Insulin & c-peptide measurements are of most value when the subject is fasting. Ideally the subject's blood glucose concentration is below 3 mmol/L. Results on non-fasting samples are extremely difficult to interpret.

The non-accredited assays are intact proInsulin, leptin, adiponectin, PARC (a chemokine) and Vitamin C.

Samples for clinical assays are sent to Cambridge University Hospital's Blood Sciences Laboratory reception. From there the request is booked into the hospital computer system (EPIC) and samples are centrifuged and separated. The serum/plasma is frozen at -20 degrees C until it is analysed.

NB : Clinical Vitamin C samples will only be accepted after prior discussion with Senior CBAL staff. Careful sample collection and processing is required to get a reliable result.

Specificity of the Insulin assay

The DiaSorin assay is designed to measure endogenous insulin. However, in common with most commercial assays, it does not detect all of the forms of insulin that are prescribed to patients. We have a good understanding of the assay specificity. Therefore, when investigating these patients it is essential to give us details of any therapeutic insulin prescribed to the patient along with the dose and time it was taken.

If the therapeutic insulin is one that is not detected by the Diasorin method we can use a back-up assay (Mercodia IsoInsulin ELISA) which has a wider specificity but a poorer working range. The Mercodia assay is considerably more expensive and labour intensive than DiaSorin so analysis will only be undertaken after discussions with CBAL. The Mercodia assay may also be the method of choice when investigating suspected accidental or intentional insulin overdose.

% Cross-Reactivity

Insulin Analog	Diasorin	Mercodia
	Liaison-XL	Iso-Insulin
Actrapid	104	140
Humulin S	124	140
Hypurin neutral porcine	128	108
Novorapid (aspart)	2	140
Humalog (lispro)	1	139
Apidra (glulisine)	0	140
Hypurin neutral bovine	88	95
Lantus (glargine)	16	93
Levemir (determir)	1	46
Tresiba (degludec)	1	44
Humulin I	123	140
Insulatard	139	128
Hypurin Isophane porcine	19	14
Hypurin Isophane bovine	140	140
HPZ	140	78

C. Parfitt *et al* 2015 PMID: 26171976

A Brief Summary Of Sample Requirements for Clinical Assays

Insulin: Serum, lithium heparin plasma, EDTA plasma. Addenbrooke's – send whole blood to the laboratory on ice promptly after collection. Projects & External laboratories. Centrifuge and separate whole blood promptly. Freeze serum/plasma at -70 degrees C until analysis. Send to CUH on dry ice. Sample volume needed = 200µl.

C-Peptide: Serum, lithium heparin plasma, EDTA plasma. Addenbrooke's – send whole blood to the laboratory on ice promptly after collection. Projects & External laboratories. Centrifuge and separate whole blood promptly. Freeze serum/plasma at -70 degrees C until analysis. Send to CUH on dry ice. Sample volume needed = 200µl.

Intact Proinsulin: Serum, lithium heparin plasma, EDTA plasma. Addenbrooke's – send whole blood to the laboratory on ice promptly after collection. Projects & External laboratories. Centrifuge and separate whole blood promptly. Freeze serum/plasma at -70 degrees C until analysis. Send to CUH on dry ice. Sample volume needed = 200µl.

Leptin: Serum, lithium heparin plasma, EDTA plasma. Addenbrooke's – send whole blood to the laboratory promptly after collection. Projects & External laboratories. Centrifuge and separate whole blood promptly. Freeze serum/plasma at -70 degrees C until analysis. Send to CUH on dry ice. Sample volume needed = 150µl.

Adiponectin: Serum, lithium heparin plasma, EDTA plasma. Addenbrooke's – send whole blood to the laboratory promptly after collection. Projects & External laboratories. Centrifuge and separate whole blood promptly. Freeze serum/plasma at -70 degrees C until analysis. Send to CUH on dry ice. Sample volume needed = 150µl.

PARC: Serum, lithium heparin plasma, EDTA plasma. Addenbrooke's – send whole blood to the laboratory after collection. Projects & External laboratories. Centrifuge and separate whole blood promptly. Freeze serum/plasma at -70 degrees C until analysis. Send to CUH on dry ice. Sample volume needed = 100µl.

Vitamin C : Deproteinised lithium heparin plasma. Please contact the laboratory for advice before collecting samples.

Turnaround Times

We currently run the insulin & c-peptide assays twice a week (Monday & Thursday). Urgent samples may occasionally be analysed with prior arrangement. PARC is run fortnightly to give clinicians time to interpret the result and adjust patient management if required. Our turnaround time for leptin, adiponectin and intact proInsulin is one month from sample receipt. In practice, results are reported a lot faster than this.

We occasionally run urgent proinsulin analysis in cases of suspected proinsulin secreting tumours. This must be requested by a senior clinician.

CBAL are the only providers of 'clinical' adiponectin & leptin assays in the UK (www.assayfinder.com).

Vitamin C is very rarely required for clinical investigations. Please contact the CBAL for further advice. If analysis is agreed then the turnaround time may be up to 1 month.

All clinical results are entered into the EPIC computer by CBAL staff before being validated by a senior CUH Blood Science Laboratory clinician. Once validated, results are transferred electronically into the patient's notes (for CUH patients) or back to the requesting laboratory.

National Severe Insulin Resistance Service (SIR)

CBAL provides assay support for this National service which is based at Cambridge University Hospitals NHS Trust. Along with provision of the Clinical assays described above we also help with the investigation of patients suspected of having anti-insulin antibodies. This highly specialist work includes analysis of gel filtration chromatography fractions and antibody precipitation techniques. Results from CBAL assays are returned to the SIR team who interpret them along with results from other investigations.

Additional support for this service is provided by Dr Richard Kay who has developed LC-MS assays for a range of insulin analogues and mutations. Clinical input for the anti-insulin antibody work is provided by Dr David Church and Dr David Halsall.

Result reporting & format

Reporting of 'clinical' samples has been described above. The sequence for reporting research assays is as follows.

CBAL staff will run the assays, check the quality control results are within specifications, then enter the results into the result spreadsheet. When all results have been entered the transcription will be checked by a second Biomedical Scientist before being passed on a Senior Biomedical Scientist for final checks and reporting.

Unless otherwise requested results will be returned on an Excel spreadsheet via email. We would appreciate an email in return to confirm that results have been received.

We are always willing to discuss unexpected or unusual results. Please send an email to cbal@addenbrookes.nhs.uk describing the problem.

Results are held securely on password-protected CUH Trust servers. We store over 10 years worth of result data. Most archived results can be retrieved quickly on request.

Reference Ranges

Clinical Assays

We are able to provide reference ranges for each of the clinical assays described above. Ranges for insulin, c-peptide, intact proinsulin, adiponectin & leptin have been derived from a local population study (Fenland Study) co-ordinated by Professor Nick Wareham.

We have data for fasting levels of insulin, c-peptide & intact proinsulin.

Reference ranges for adiponectin and leptin are corrected for the subject's BMI.

NB it is essential to be aware that results from different organisations/methods may not be directly comparable to results from CBAL assays.

Current Reference Ranges for CBAL Clinical assays

Insulin : Measured in 496 subjects, aged 40-65 years, with normal oral glucose tolerance test and normal BMI (22.5-27.5) the reference range was determined to be 'Less than 80 pmol/L'.

C-Peptide : The Reference Range for fasting adult samples was 174 - 960 pmol/L

Intact ProInsulin : Measured in 495 subjects, aged 40-65 years, with normal oral glucose tolerance test and normal BMI (22.5-27.5) was 'Less than 7 pmol/L'.

Leptin

For Adults (>18years of age)

Gender	BMI (kg/m^2)	5th – 95th centile (ng/ml)	n	Median (ng/ml)	Minimum (ng/ml)	Maximum (ng/ml)
Male	<25	0.4 – 8.3	278	2.5	0.1	22.8
	25-30	1.5 – 13.0	375	4.7	0.5	26.3
	30-35	4.2 – 26.0	98	9.5	2.1	36.7
	>35	7.8 – 31.7	8	15.5	7.8	31.7
Female	<25	2.4 - 24.4	535	8.9	0.2	45.8
	25-30	8.6 – 38.9	348	18.9	3	65.7
	30-35	14.9 – 60.2	126	32.3	8.1	79.1
	>35	22.7 – 113.6	60	52.4	11.9	137.4

Results are taken from an East Anglian adult population study.

There are no paediatric reference ranges available for Leptin.

Adiponectin

For Adults (>18years of age)

Gender	BMI (kg/m^2)	5th – 95th centile (mg/ml)
Male	<25	2.6 – 12.6
	25-30	2.4 – 10.6
	30-35	2.8 – 9.9
	>35	insufficient data
Female	<25	4.4 – 17.7
	25-30	3.5 – 15.5
	30-35	2.6 – 14.9
	>35	2.6 – 17.1

Results are taken from an East Anglian adult population study.

There are no paediatric reference ranges available for Adiponectin.

PARC

Measured in a cohort of 105 normal adult volunteers & subjects without Gaucher disease the reference range was determined to be ‘up to 150 ng/ml’.

Vitamin C

An in-house study in 1995 determined the reference range to be 34-114 µmol/L.

Reference Ranges for Non-Clinical Assays

Whilst CBAL uses the best assays available for each study it is unlikely we will be able to provide a reliable reference (normal) range for most of the tests.

The primary reason for this is that we are unable to source samples from the hundreds of 'normal' subjects required to generate a statistically valid reference range. Many reference ranges may also be influenced by the sex or age of the subject. This would require additional investigations and considerable cost. Our in-house assay development data will enable us to generate an approximation of 'normal' concentrations found in human serum. This is often sufficient.

Some of the more reliable research kit manufacturers provide brief information on the 'expected range' of results from normal subjects. Whilst this is helpful it does not equate to a true 'reference range' as the number of subjects investigated is typically small (n=30).

Many manufacturers provide no information at all.

Method development & Evaluation

The instrumentation in CBAL enables us to perform a wide range of biochemical assays. We always try to use high-quality commercially-sourced assay kits whenever possible as they are likely to have been fully developed and evaluated by the kit manufacturer.

There will be times when there are no commercial assays available or those that are available are unsuitable. In these cases we will attempt to develop assays ourselves.

CBAL does not have resources to commission the generation of antibodies for a project. Therefore, most of our in-house method development will be done with commercially-available antibodies and 'calibration' materials.

Sourcing suitable antibodies is a big challenge. If assay development kits (for example DuoSets from R&D Systems) are available this will be the preferred option. The alternative option of selecting two antibodies at random from commercial catalogues and hoping they will work as a 'pair' is extremely unpredictable. In our experience this approach works less than 20% of the time. We have noticed that the same antibody is often available from different vendors under different product codes.

Recombinant proteins are frequently used as assay calibration materials. In our experience the recombinant proteins sold as part of assay development kits (DuoSets) tend to work well. However, not all recombinant proteins are guaranteed to behave in the same way as native proteins found in human samples.

Once we have sourced suitable antibodies and calibration materials we set about developing the assay.

The following options are evaluated before we can decide whether the assay is suitable.

(a) Analytical Platform

Virtually all of CBAL's immunoassay development work is done on the MSD platform. Other platforms such as Luminex or DELFIA may be considered if necessary.

(b) Cost

The cost will be assessed at the beginning of the project. If the projected costs of the assay development and evaluation exceeds the researcher's budget the project will be abandoned.

(c) Preliminary Experiments

In these experiments we will attempt to optimise the concentrations of capture and detection antibody used for the assay. This is done with the aid of a standard curve prepared from the Recombinant biomarker of interest.

By the end of the preliminary experiments we will have an idea whether the assay is going to work. The quality of the assay might be enhanced by changing a number of factors within the assay such as assay diluents.

(d) Secondary Experiments

Once the raw assay has been created we look to optimise it further to ensure that it is suitable for the samples undergoing testing. We have serum and plasma pools that are used to ensure the working range of the new method is appropriate. Most biomarkers are present at low concentration so sample pre-dilution is not required. Occasionally sample dilution will be required to get results into the assay range – this will become part of the optimisation process.

Our preferred incubation times are 2 hours with sample, 1 hour with detection antibody and 30 minutes with TAG reagent. Very occasionally we might need to change the incubation times for various steps in the assay.

(e) Successful optimisation from (c) and (d) takes us onto the assay performance evaluation stage. We look at the lower limit of detection of the assay, the working range, concentration of the biomarker in a small number of paired lithium heparin plasma/EDTA plasma & serum samples from 'normal' male and female donors, within-batch reproducibility (samples with low, medium and high concentration of the biomarker run multiple times in a plate). We also look at recovery of recombinant biomarker spiked into the human sample matrix (spike recovery) and the results from human samples diluted in assay diluents (linearity on dilution). Failure of either of these last two assessments may indicate a serious problem with the assay such as binding protein interference or non-specificity.

(f) Longer-term performance assessment will include a study where Quality Control samples with low, medium and high concentrations are run over multiple batches. The reproducibility of the results is assessed. We aim for coefficients of variation below 10%.

We also attempt to assess the stability of the biomarker if samples are left for an excessive time at room temperature or 4 degrees C rather than being frozen at -20 degrees C or below. We also look at the effect of multiple sample freeze-thaw cycles on the biomarker concentration.

We are often asked for information on stability of biomarkers during long-term storage (>1 year). Unfortunately, this information is not readily available. Neither is information on the stability in whole blood samples that have not been centrifuged within an appropriate time.

(g) We attempt to assess the effects of interfering factors on the assay. Manufacturers usually give an indication of the cross-reactivity of their antibodies with biomarkers of similar structure. We also try to assess the effects of haemolysis, lipaemia & icterus on the assay. We will also attempt to check whether abnormally high concentrations of serum proteins such as immunoglobulins and rheumatoid factor have an adverse effect. We are aware of the potential interference from heterophyllic antibodies on two-site immunoassay. Some blockers are present in the reagents but any unexpectedly high result may require further investigation.

Typically, we will spend up to a month undertaking the assay development and evaluation. This may be considerably longer if we encounter difficulties. At the end of the assay development phase a written report is prepared and the researcher is told whether the assay is suitable for the project to go ahead.

Accreditation

CBAL currently has limited ISO 15189 accreditation with the UKAS scheme (www.ukas.com). Only two assays are within scope for clinical diagnostic purposes (insulin & c-peptide).

A further four assays are validated to a high standard and used for clinical diagnostic purposes (proinsulin, adiponectin, leptin & PARC). For organisational reasons these assays have not been submitted to UKAS for accreditation.

The remainder of the assays do not have any accreditation status. However, you can be assured that we will perform the assays to the highest standards and ensure strict quality assurance systems are in place before reporting results.

Inevitably, there will be assays that are difficult to perform and assay kits that do not perform to the expected standard. We will always try to discuss potential assay problems with the researcher before undertaking or during the analysis process.

Contracts & Documentation

We attempt to keep paperwork to a minimum. For most small-scale projects we are happy to keep a record of email exchanges and use these as verification that the study is going ahead. We will have information regarding the tests required, the prices we will charge and the destination of the result files & invoice.

If a more formal agreement is required we have a standard document that can be modified to cover different circumstances as required. The document is signed by CBAL and the researcher and each party has a copy for their records.

We are getting many more requests to fill in registration documents for research studies falling under MHRA guidelines. Completing these documents is time-consuming and most of the information required is not available for CBAL as we do not work under GLP guidelines.

Test Pricing

CBAL is expected to recover its staff and operational costs and continue to make a small profit for the Cambridge Biomedical Research Centre (BRC).

The true cost of running each assay has been calculated carefully. The calculation assumes that reagents will be used at 90+ % efficiency. If we are asked to perform sample numbers that will result in very inefficient reagent usage we may have to pass this additional cost on to the researcher.

If we are asked to create a 'novel' assay we expect the researcher to contribute at least 50% of the start-up costs. This will be discussed with the researcher before starting the project. Occasionally, the method development will fail. In these cases the payment still applies.

CBAL operates a 3-tier pricing system. Lowest prices will be offered to groups affiliated to the Cambridge BRC. The second tier pricing is offered to other Academic Institutions in the Cambridge Area and around the UK. The highest pricing will be offered to Commercial Organisations and pharma-sponsored research studies.

Occasionally, CBAL might be able to offer some discounts for particularly large studies.

All prices quoted are excluding VAT. Please inform CBAL if you need VAT adding to the invoice.

Quotes (written or email) for assay work can be obtained by contacting CBAL.

We have decided not to publish a price list as it will rapidly become out of date.

Prices are usually increased by approximately 5% annually.

Quality Assurance & Quality Control

All assays performed in CBAL have a high level of quality assurance. The following quality procedures are in place to ensure the accuracy and reproducibility of the results we issue.

Within-Batch Quality Control (QC)

Each batch will contain a minimum of two QC samples which are analysed at the beginning and end of each run. Where possible QC samples are treated in exactly the same way as 'unknowns'. Therefore, the results obtained reflect the quality of results obtained in the rest of the assay.

Quality control samples are sometimes provided with the kits we use. Also, there are also commercially available serum and urine solutions that contain appropriate concentrations of many commonly-measured biomarkers. However, many of the assays performed in CBAL do not have a readily available QC so we create our own. These QCs are usually prepared from pools of human serum/plasma/urine or from these pools supplemented with recombinant biomarkers of interest. We aim to prepare good volumes of at least three pools with low, medium and high concentrations of the biomarker under test. These pools are then split into assay-sized aliquots and stored at -80 degrees C. Fresh QC aliquots are used for each batch. When a new batch of QC material is prepared we will analyse it in parallel with the current batch, ideally for a minimum of ten runs to establish new acceptance limits.

Results from internal QC samples are used to determine whether an assay has 'worked' and whether results can be released. This is done by comparing results for all of the QC samples with their respective target ranges. If all results are within their target range the batch is deemed to be acceptable. It is often useful to compare results from the beginning of the run with those from the end. If there is significant difference in results from beginning to end there may be assay 'drift'. The presence of 'drift' may be sufficient to reject a batch.

Another form of QC used in each batch is monitoring of the 'signal' obtained for each of the assay standards. A significant difference from the expected signal may indicate that there has been a problem with the assay.

Unknown samples are generally analysed in duplicate with the mean result being reported. Our analysis software will calculate the coefficient of variation (CV) of these two results. For a valid result we aim to achieve a CV of less than 10% (this may vary according to the assay type and the biomarker concentration in the samples). If the majority of samples in an assay have CVs greater than 20% there may be a technical problem. Assays on the fully automated Diasorin Liaison XL and Siemens Dimension EXL analysers are performed in singleton.

Between-Batch Quality control

We frequently undertake large studies where samples are analysed over multiple batches. Our between-batch QC procedures aim to ensure that results from all batches are comparable. This is done by reviewing the QC data for each batch against the previous batches and the QC target ranges.

All assay QC data is stored in an Excel spreadsheet along with the 'signal' for the assay standards and any other relevant information. We use this data to determine the batch-to-batch variability of the QC. We aim for CVs of less than 10% but will occasionally accept values up to 15% if the assay is particularly difficult to perform.
QC is reviewed at the end of each project. Providing there are no concerns about assay reproducibility the results are released. Poor QC performance may require re-evaluation of the analytical method and possibly reanalysis of the samples.

One important feature of between-batch QC data is the ability to see differences in assay performance when studies are performed months apart. Our within-batch QC aliquots are designed for use over an extended period (1-2 years) provided degradation can be avoided. Therefore, a significant change in QC results over a 6-month period may indicate a problem with the assay. Unfortunately, we have had several instances where kit reagents or calibrators have been changed without notice by the manufacturers. This is out of our control but we attempt to inform the researcher once we have identified the problem.

Eternal Quality Assessment Schemes (EQAS)

EQAS schemes are designed to improve consistency for all laboratories returning results for a particular assay. The information from these schemes often clearly demonstrates the variability of results for commonly requested tests analysed on different instruments, analytical methods and calibrators.

Almost all of the more esoteric assays performed by CBAL are not covered by an EQAS scheme. Assays which have a suitable scheme need samples to be analysed and results submitted on a regular basis. This does not fit in with the infrequent use of the majority of the assays offered by CBAL.

Much of CBAL's work does not lend itself to participation in EQAS schemes. Results need to be returned to the scheme co-ordinators regularly. This does not fit in with the 'sporadic' nature of CBAL's workload. Also, many of the more esoteric assays are not covered by EQAS schemes.

CBAL does participate in the EQAS Scheme for insulin & c-peptide. Samples are distributed from a laboratory in Guildford. The samples are sent to every UK laboratory performing 'clinical' insulin & c-peptide measurements. Results are returned to the central laboratory who analyse the data & issue reports.

These reports show how we are performing in comparison to our peers and also the variability of results from laboratory to laboratory. These variations are often dependent on the analytical method used. Surprisingly, there is often considerable variability even for relatively well-characterised assays such as insulin & c-peptide.

In the event of continual poor performance the scheme organisers have the power to stop a laboratory performing the test. Fortunately, CBAL's performance statistics for this scheme have always been excellent.

On request, CBAL can also join other EQAS schemes. We are currently registered to schemes for the measurement of serum cholesterol, triglycerides, HDL, ferritin & CRP. If this is additional service is required for a study it must be discussed at an early stage as it can take some time to register with a scheme. Additional costs incurred for registering with these schemes are passed on to the researcher.

CBAL has 'informally' analysed samples from EQAS schemes for NEFA, 3-OH Butyrate and Vitamin C. All CBAL results were shown to be in consensus with other laboratories.

Quality Control Review

Quality Control is reviewed at all stages of the analytical process. No results are issued until the QC is satisfactory. QC issues are discussed at the CBAL monthly staff meeting and , if needed, at the quarterly CBAL management meeting.

CBAL EQAS data may be presented at the CUH Blood Sciences Management meeting.

Turnaround Times for Research Studies

Turnaround times for 'cinical' samples are described in the Clinical Assays section.

For research samples we usually give an estimate of turnaround times when the study is set-up. This may be days for a simple project and up to 6 months for a more complex project where we are measuring 50+ analytes on thousands of samples.

We are increasingly finding that analysis is delayed because the Principle Investigator is unable to access the samples due to ethics or other regulatory issues. Obviously, this is out of CBAL's control but we are willing to provide documents confirming a collaboration if that helps to speed up the process.

Occasionally CBAL will have difficulty obtaining the required kits or reagents. This may be due to procurement issues, supplier issues or misplaced deliveries. Once we are aware of a problem we will do our best to sort out the issues and keep the researchers informed of the delays and progress in resolving them.

Requests for 'urgent' analysis are taken on a case-by-case basis. We will always try to help if possible but there may be cost implications due to inefficient use of reagents and staff time.

Collaborations & Publications

One of the criteria used to determine the success of CBAL is the number of co-authorships and acknowledgements we receive in peer-reviewed journals. Therefore, it is essential that CBAL is at least acknowledged in any publication using our assay data. Wording should be along the lines of 'xxx assays were performed by the NIHR Cambridge BRC Core Biochemical Assay Laboratory'. Unfortunately, we are unable to police these acknowledgements and have to trust researchers to include them. It is very likely a significant number of papers are published without them. This may have an adverse effect on any future funding for CBAL. We are always willing to contribute to and proof-read manuscripts before submission to publishers.

Co-authorships for members of CBAL run into the 50's over the past 10 years. Acknowledgements could be well over 100 but we do not hold this information.

Publications have covered such diverse research areas as Obesity & Metabolism, Diabetes, Cancer diagnostics, HIV Prognostic markers, Bone disease, Renal function, and Gaucher Disease.

CBAL is proud to have worked with many prestigious departments and organisations across the UK, Europe and further afield.

In the local area CBAL has worked with many of the Cambridge University departments based on the Addenbrooke's Site, including the Institute of Metabolic Science, MRC Epidemiology, Department of Medicine, Department of Biochemistry, Department of Obstetrics & Gynaecology, Department of Paediatrics, Department of Surgery & the Department of Regenerative Medicine.

CBAL has also worked with researchers from Royal Papworth Hospital, MRC Laboratory of Molecular Biology (LMB), Babraham Institute, Sanger Institute, Anglia Ruskin University and the CIMR.

CBAL had performed analysis for many hospital and university based research departments around the UK. These include Norwich, Southampton, York, Dundee, Aberdeen, Bristol, Kings College London, University College London, Liverpool, Newcastle, Cardiff & Edinburgh.

Requests for clinical sample analysis of leptin & adiponectin come from many hospital departments round the UK.

CBAL has analysed samples in collaboration with organisations in Turku (Finland), Copenhagen (Denmark), Brescia (Italy), Dublin & Galway (Eire) & Brussels (Belgium) .

In addition, CBAL has worked with several start-up companies to help them achieve their goals. CBAL has a long history of working with ProteinLogic on their 'Immiprint' technology. CBAL has analysed thousands of samples for this company for an extended range of biomarkers, assays for many of these were developed in CBAL. CBAL has also helped with development of a point-of-care device for the measurement of many 'commonly-requested' biochemistry tests and the development of a new group of tests with the aim of reducing the number of biopsies required for the diagnosis of prostate cancer. Many more projects are currently at the design stage.

A brief summary of some publications that contain CBAL assay data

(with PubMed Index number).

Paradoxical elevation of high-molecular weight adiponectin in acquired extreme insulin resistance due to insulin receptor antibodies.PMID: 17325257

Plasma adiponectin as a marker of insulin receptor dysfunction: clinical utility in severe insulin resistance. PMID:18299442

Urinary insulin-like growth factor 2 identifies the presence of urothelial carcinoma of the bladder. PMID:19040529

A genome-wide association study reveals variants in ARL15 that influence adiponectin levels. PMID: 20011104

Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals. PMID: 20018283

Increased maternal Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) associated with older age at diagnosis of Type 1 diabetes in offspring. PMID:21105490

Analysis of molecular forms of urine Retinol-Binding Protein in Fanconi Syndrome and design of an accurate immunoassay. PMID:22120727

Bladder cancer diagnosis and identification of clinically significant disease by combined urinary detection of Mcm5 and nuclear matrix protein 22. PMID:22792272

Leptin levels in children and adults with classic galactosaemia. PMID:23430559

Cannabis use is associated with increased CCL11 plasma levels in young healthy volunteers. PMID:23820464

Attainment of Metabolic Goals in the Integrated UK Islet Transplant Program With Locally Isolated and Transported Preparations. PMID:24119216

Home Urine C-Peptide Creatinine Ratio Can Be Used to Monitor Islet Transplant Function. PMID 24623023

Reusable, Robust, and Accurate Laser-Generated Photonic Nanosensor. *Nano Lett* May 20, 2014

Insulin-like peptide 5 is an orexigenic gastrointestinal hormone.PMID: 201411413.

Characterising the association of latency with α_1-antitrypsin polymerisation using a novel monoclonal antibody. PMID:25462157

Effects on vitamin D, bone and the kidney of switching from fixed-dose tenofovir disoproxil fumarate/emtricitabine/efavirenz to darunavir/ritonavir monotherapy: a randomized, controlled trial (MIDAS). PMID:26460504

Heterogeneity of glucagonomas due to differential processing of proglucagon-derived peptides. PMID:26693280

Assessment of Renal Injury in Patients Undergoing Elective EVAR Using Urinary Neutrophil Gelatin-Associated Lipocalin, Interleukin 18, and Retinol-Binding Protein. PMID:27707982

The Prostate Health Index adds predictive value to multi-parametric MRI in detecting significant prostate cancers in a repeat biopsy population. PMID:27748407

INNOVATE: A prospective cohort study combining serum and urinary biomarkers with novel diffusion-weighted magnetic resonance imaging for the prediction and characterization of prostate cancer. PMID:27769214

Novel Mechanism for buffering dietary salt in humans: Effects of salt loading on skin sodium, Vascular Endothelial Growth Factor C, and blood pressure.PMID:28974570

Extracellular Lactate: A novel measure of T Cell proliferation.PMID:29288205

Frequent Monitoring of C-peptide Levels in Newly Diagnosed Type 1 Subjects Using Dried Blood Spots Collected at Home. PMID:29860430

The plasma biomarker soluble SIGLEC-1 is associated with the type I interferon transcriptional signature, ethnic background and renal disease in systemic lupus erythematosus. PMID:30053827

Assessment and Management of Anti-insulin autoantibodies in varying presentations of Insulin Autoimmune Syndrome. PMID:30085133

Low-dose interleukin-2 in patients with stable ischaemic heart disease and acute coronary syndromes (LILACS): protocol and study rationale for a randomised, double-blind, placebo-controlled, phase I/II clinical trial. PMID:30224390

Associations of vomiting and antiemetic use in pregnancy with levels of circulating GDF15 early in the second trimester: A nested case-control study. PMID: 30345390

A New Estimate of the Glomerular Sieving Coefficient for Retinol-Binding Protein 4 suggests it is not freely filtered. PMID:31384698

Early safety of tenofovir alafenamide in patients with a history of tubulopathy on tenofovir disoproxil fumarate: a randomized controlled clinical trial. PMID: 31679186

Poster Presentations

A sensitive and precise automated immunoassay for PARC – A serum Chemokine marker of Gaucher Disease.(presented at European Gaucher Disease Conference, Barcelona 2004 and Focus, Glasgow 2005)

Hypoglycaemia due to an insulin binding antibody in a patient with an IgA kappa myeloma. (presented at Focus, 2006)

Urinary NGAL does not correlate with renal dysfunction in polycystic kidney disease. (presented at ASN, 2011)

Case of anti-insulin antibodies affecting glycaemic control in a diabetic. (presented at IBMS, Birmingham, 2011)

Effects of renal tubular dysfunction on bone (presented at IAS, 2013)

Effects on Vitamin D and bone of switching from Atripla to Darunavir/Ritonavir (presented at CROI, 2014)

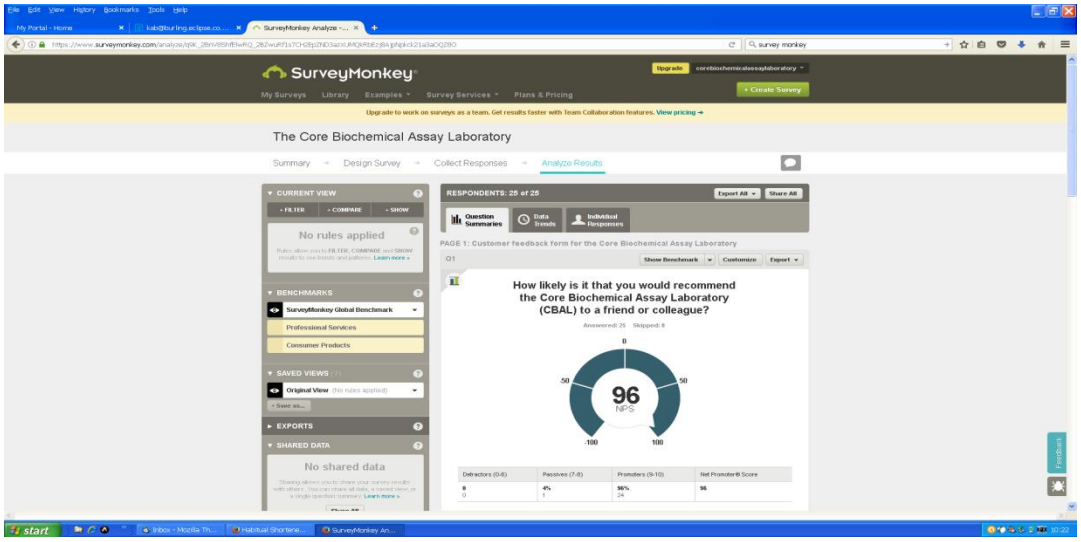
Evaluation of a novel method to detect residual ß-cell function by dried blood spots in children and adolescents with a recent diagnosis of type 1 diabetes (presented at International Society for Pediatric and Adolescent Diabetes, Valencia, Spain 2016).

INNOVATE combIning advances in imaging with biomarkers for improved diagnosis of aggressive prostate cancer (presented at NCRI Cancer Conference Liverpool 2016).

Low bone mineral density in older people with HIV: The renal-bone axis and art (presented at CROI 2020)

Satisfaction Survey

We occasionally run a 'satisfaction survey'. The link to this survey will be found at the bottom of email signatures sent from CBAL. Please take the time to fill in the survey and add suggestions where we can improve the service we offer.



We are very proud to report that on a previous survey we previously earned a 96% approval rating.

Frequently Asked Questions (FAQs)

How long will the sample analysis take ?

We always aim to process samples as quickly as possible. Often there will be delays if we need to order kits or reagents. The number of samples and the complexity and number of tests required will also influence the analysis time. We can often process 100 samples for 1 or 2 biomarkers within a week of receipt. Thousands of samples for multiple biomarkers may take 3 to 6 months to complete. High workload and reduced staffing levels may also influence the turnaround time.

Can I help with the sample analysis ?

Unfortunately, we do not have the staff resources to allow us to supervise untrained staff running our assays. However, we are always willing to let people come to watch the assays being performed while we explain the techniques in greater detail.

Will my sample be analysed in singleton or duplicate ?

Most simple chemistry tests (glucose, triglycerides, cholesterol etc...) are analysed in singleton. Automated immunoassay on the Diasorin Liaison, Siemens Dimension and Randox Daytona are also analysed in singleton.

For Microtitre plate immunoassay (ELISA, MSD & Luminex) we recommend sample analysis in duplicate. Singleton analysis may be undertaken at the researcher's request if they have insufficient sample for duplicates or if the cost of duplicates is too high. We would not recommend this approach.

How can I deliver samples to CBAL ?

Please inform the laboratory that you are going to deliver samples. CBAL staff will inform you of a suitable delivery time. Please send the samples to the laboratory in appropriate packing. Usually samples are frozen in dry ice in a suitably insulated box. Samples may be delivered by hand or via a courier. It is your responsibility to ensure and packaging, labelling and transportation regulations are met. Maps showing the location of CBAL are available on request.

If you are delivering samples by courier we suggest sending them them no later than Wednesday to ensure they are received by mid-day Friday. We have no staff available to receive packages over the weekend. We will send an email to confirm samples have arrived safely.

How can I get more information about the analytical methods you plan to use ?

For well-established CBAL assays we have 'assay summary' sheets. These are sent to the researcher on request. They contain a brief explanation of the method, details of the reagent manufacturer, assay precision, assay working ranges and possible interfering factors. The detail in these summaries is usually sufficient for publications but if more information is required then please contact the laboratory. We can supply a less-detailed summary for new assays.

What happens to my samples when the work is complete ?

When the work is complete there are three options

(a) arrange with CBAL to take the samples away. Samples can be collected from our laboratory during our core working hours. We are unable to send samples by courier. However, we are happy for the researcher to arrange for a courier to collect the samples. BioCair offer a good service.

(b) store the samples in CBAL in case additional tests are required. We have very limited storage space. We can store samples at -80 degrees C for up to 3 months. After this time the samples will be discarded.

(c) ask CBAL by email to discard the samples in accordance with local rules. We can supply written confirmation that the samples have been discarded if required.

How am I charged for the work ?

We do not issue invoices for work until it is complete and the researcher is satisfied with the results. We then prepare invoicing details for the CUH Finance Department who issue the invoices. We also send a cover sheet which gives additional information about the work we have undertaken. We find that each organisation has its own requirement for invoicing. If we need to quote a purchase order number or Grant Code please ensure it is given to us. For long-term and large studies we may have to issue invoices at regular intervals to cover the cost of reagent purchases.

Very occasionally we might be able to issue an invoice before the work is complete. Please contact the Laboratory Director to discuss.

Is CBAL accredited ?

CBAL has insulin & c-peptide assays in scope for UKAS ISO15181 accreditation. It does not have accreditation for any of the other assays. Please note that the majority of kits used in CBAL are branded as ‘research use only’.

When should I contact CBAL about analyzing samples for a study?

Ideally, we would like some input at the study design stage. This ensures that the appropriate sample are collected (this covers sample types, aliquot tubes, aliquot volumes & labelling) and that suitable assays are available.

We are happy to discuss studies after samples have been collected. However, you should be aware that some sample types may be unsuitable for specific tests. We can discuss studies in person, by email or phone.

Do you only run the assays described on the website or in this brochure?

No, these are examples of the assays we have undertaken in the past or are available on the equipment we have. If you are interested in running assays we have not mentioned please do ask. We will try our best to assist you.

My samples are not in your recommended tubes will you still be able to analyse them ?

We are able to modify our systems to use a variety of tube types. As a last resort we might have to transfer the sample to a recommended tube type, this may result in a minimal additional handling charge. But please talk to us and let us see an example of the tubes.

Can I ask what might be a basic question about some work you have done for me?

Please do ask us. We want to help you and your research as much as possible. We aim to supply a clear and concise answer to all questions put to us.

I have 30ul of sample and want to do a wide variety of tests. Can you assist?

There is a limit to what we are able to achieve from a small sample and this will depend upon the assays required. We can work with you to obtain as many results as possible. Occasionally, logistics dictate that we run certain groups of tests together.

Do you offer work experience placements ?

Unfortunately, we do not have the resources to enable us to offer this.

If your question is not answered anywhere in this brochure - please do contact us....

Thank You's & Acknowledgements

Thanks must go to the talented, hard-working and dedicated CBAL staff (past & present) who have made the laboratory so successful and well-regarded. Thanks also to Mr Ian Halsall, Mrs Fiona Tulloch & Mr Kevin Taylor who developed many of the insulin assays and managed the laboratory during its formative years.

Thanks also to Professor Sir Steve O'Rahilly, Mr Colin Carr & the current and ex- members of the CBAL management team for their guidance and advice over the last 12 years.

Normal Range data for insulin, c-peptide, intact proinsulin, leptin & adiponectin were derived from Professor Nick Wareham's Fenland & Ely studies.

Dried blood spot photographs were supplied by the ITAD research group in Oxford.

Some illustrations were taken from the MesoScale Discovery, Randox, DiaSorin & PerkinElmer websites.

Document prepared by Keith Burling, Director of CBAL during the UK Lockdown, Spring 2020.

The emergence of Covid-19 is likely to influence the assay requirements of researchers for years to come. CBAL is well placed to meet these requests. We have modified our assay protocols to ensure there is minimal risk to staff handling coronavirus positive samples and our assay manufacturers are expanding their assay ranges to cover tests that are likely to be needed.

An alphabetical list of biomarker assays currently available in CBAL

1-25 OH Vitamin D	25-OH Vitamin D	3-OH Butyrate	Abeta 38-40-42
Adiponectin	Afamin	AFP	Albumin
Aldolase	Aldosterone	Alkaline Phosphatase	Alpha klotho
Alpha-1-Antitrypsin	ALT	Amylase	Androstenedione
Angiopoietin-2	Apolipoprotein A-I	Apolipoprotein A-II	Apolipoprotein B
Apolipoprotein E	AST	BDNF	Bile Acids
Bilirubin	CA 15-3	CA 19-9	Caeruloplasmin
Calcium	CD117	CD120b	CD121b (IL-1 rII)
CD137 (TNFRSF9)	CD14	CD150 (SLAM-F1)	CD152 (CTLA-4)
CD163	CD166 (ALCAM)	CD170	CD222
CD239 (BCAM)	CD25 (IL2R alpha)	CD26	CD263 (Trail R3)
CD27 (TNFRSF7)	CD282 (TLR-2)	CD32 b/c	CD324 (E-Cadherin)
CD33	CD35 (Comp rec-1)	CD40 (TNFRSF-5)	CD40L (TNFSF5)
CD50 (ICAM-3)	CD56 (NCAM)	CD62-L (L-selectin)	CD85j (ILT-2)
CD87 (uPAR)	CDw329 (Siglec 9)	CEA	Cholesterol
Choloride	CK	CK-MB	Comp Factor H
Cortisol	C-Peptide	Creatinine	CRP
CRP (high sensitivity)	CXCL-10 (IP10)	CXCL-9	DHEAS
DLK-1	EGFR	Estradiol	Ferritin
FGF-21	FGF-23	FLT-1	Folate
Free T3	Free T4	Fructosamine	FSH
Gamma GT	GDF-15	Ghrelin (active)	Ghrelin (total)
GIP	GLP-1 (active)	GLP-1 (total)	Glucagon
Glucose	Glutamine	Glycated Albumin	Glycerol
Glycomark	Granzyme A	Granzyme B	Growth Hormone
Haptoglobin	HbA1c	HDL	Hepcidin
Hyaluronic Acid	ICAM-1 (CD54)	IFN gamma	IgA
IgG	IgM	IGFBP-3	IGF-I
IL-1 beta	IL-10	IL-13	IL-2
IL-4	IL-5	IL-6	IL-8
Inorganic Phosphate	Insulin	Intact Proinsulin	Iron
IsoInsulin	Lactate	LDH	Leptin
LH	Lipase	Lipopolysaccharide-BP	Lipoprotein (a)
Lp-PLA2	Magnesium	MCP-1	MCP-4
MDC	MIP-1 alpha	MIP-1 beta	MMP-1
MMP-10	MMP-12	MMP-3	MMP-7
MMP-8	MMP-9	NEFA (FFA)	NT-pro BNP
Orosomucoid	Osteocalcin	Osteonectin	Osteoprotegerin
Ox-LDL	PAI-1 (Serpin E1)	PARC	PD-1
Pentraxin-2	PLAUR (uPA)	PLGF	Potassium
Progesterone	Prolactin	PSP-94	PYY
Renin	Resistin	SAA (Amyloid A)	Salivary Cortisol
Salivary DHEAS	Serpin A4 (Kallistatin)	Serpin G1	SHBG
SIK-1	Sodium	TARC	Testosterone
TIBC	TIM-3	TIMP-1	TNF alpha
TNFRSF25 (DR3)	Total Protein	Total T3	Total T4
Transferrin	Triglycerides	Troponin I	TSH
Urea	Uric Acid	Urine Albumin	Urine C-Peptide
Urine RBP	Urine Total Protein	VCAM-1 (CD106)	VEGF
VEGF-R2	Vitamin B12	Vitamin C	ZnT8 antibody

CBAL Enquiry and Submission Checklist

Can we help you?

- ❑ Contact CBAL preferably by email (see below) with the details of:
    1. Tests required
    2. Sample type
    3. Number of samples
    4. Timeframe of delivery

Sample collection and storage conditions can be specific to a test. It may be beneficial for you to contact us before you start.

- CBAL staff will advise on the most appropriate method(s) of analysis, sample collection requirements, storage and transport conditions, an estimate a cost for the work and give an indication of when the work can be completed by.

Are you ready to proceed?

- Confirm with CBAL by email
- Ensure finance is in place and generate a PO number for the invoice (if needed)
- Locate & prepare the samples to be sent. Samples need to be clearly identified with a printed label or hand-written label using ink that will survive storage below -70^{o}C and subsequent thawing (some inks will smudge when the contents of the tube are thawed).
- Place in a plastic or cardboard sample box
- Create a spreadsheet with the following headers

Sample Identification	Box Number	Box Position	Test 1	Test 2	Test 3	Test n	Comments
1							
n							

- Adjust the spreadsheet accordingly if you have other information you would like to include such as time points or visit number for example.
- Remember to indicate whether you wish to collect your samples at the end of analysis. CBAL can store samples for up to 3 months post analysis. After this time the samples may be discarded by CBAL following local rules.
- Email the spreadsheet to CBAL (cbal@addenbrookes.nhs.uk)
- Bring the samples to CBAL or arrange delivery with a courier company

What next?

CBAL will analyse the samples as quickly as possible. Results will be emailed once analysis is complete (unless there is a reason for partial release of results)

Please confirm receipt of results by emailing back

We would be grateful if you could fill in the survey attached to the email signature.