Frequently Asked Questions

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1. COMMERCIAL INFORMATION

1.1. How can I place an order?

Any Purchase Order can be sent by email, by phone or by fax to our Customer Service Department at the address customer.service@diasource.be.
We encourage you to mention the article number and the name of the ordered product.

1.2. How can I obtain a price?

Any price request can be addressed to our Customer Service at the address customer.service@diasource.be

1.3. Does DIAsource provide the kit components separately?

It is possible to purchase some components separately to the address customer.service@diasource.be

1.4. How can I address a complaint?

Any **technical** complaint can be sent to our Technical Support Department at the address products.support@diasource.be.

Any **administrative or logistical** complaint can be sent to our Customer Service Department at the address customer.service@diasource.be

Any complaint **linked to automates** and instrumentation can be sent to our Instrumentation Department the address instrumentation@diasource.be.

The issue will be registered in our Complaint Management System and the acknowledgment of receipt including the identification number of the complaint will be communicated within 24h.

We thank you to provide all detailed information: the reference of the affected product, the lot No, the raw data (calibration curve, controls data, samples), any pictures if needed, the protocol used (temperature, sample type, dilutions used, washing technique, reading fitting, storage and preparation of the reagents…).

2. REGULATORY INFORMATION

2.1. Do the DIAsource kits contain dangerous substances?

This information can be found in the corresponding Material Field Data Sheet (MSDS) of the kit that can be downloaded on DIAsource website.

2.2. Are the DIAsource assays CE mark and/or FDA approved?

DIAsource offers a majority of CE-marked kits but also offer a series of assays for Research Use Only (RUO). Moreover, our 25OH Vitamin D Total Elisa (#KAP1971) is FDA approved.

2.3. *May the DIAsource assays be sold in Canada?*

The DIAsource products available for the Canadian market are clearly identified in our catalog with a red star "* can be sold to Canada" . The complete list of products is also available on the website www.mdall.ca.

3. TECHNICAL INFORMATION

3.1. *How can I receive a technical information?*

Any technical request can be sent by email to our Technical Support Department at the address products.support@diasource.be.
The acknowledgment of receipt or a complete answer will be communicated to you within 24h.
A specific Form can also be completed and sent via the section "Contact" of our website.

3.2. *What is the principle of an immunoassay (ELISA – RIA – IRMA)?*

Immunoassay's use the basic immunology concept of an antigen binding to its specific antibody, which allows detection of very small quantities of antigens such as proteins, peptides, hormones, or antibody in a fluid sample.

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

Radioimmunoassay (RIA) is a very sensitive in vitro assay technique used to measure concentrations of antigens (for example, hormone levels in blood) by use of antibodies. As such, it can be seen as the inverse of a radio binding assay, which quantifies an antibody by use of corresponding antigens. A known quantity of an antigen is made radioactive, labeled with 125-I. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two specifically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled antigen from the serum to compete with the radiolabeled antigen for antibody binding sites. As the concentration of unlabeled antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free(unbound) antigen remaining in the supernatant is measured using a gamma counter.

Immunoradiometric assay (IRMA) is an assay that uses radiolabeled antibodies. It differs from conventional radioimmunoassay (RIA) in that the compound to be measured combines immediately with the radiolabeled antibodies. In IRMA, the antibodies are labeled with radioisotopes which are used to bind antigens present in the specimen. When a positive sample is added to the tubes, radioactively labeled, antibodies bind to the free epitopes of antigens and form

an antigen-antibody complex. Unbound labeled antibodies are removed by a second reaction with a solid phase antigen. The amount of radioactive remaining in the solution is direct function of the antigen concentration.

3.3. What are the performance characteristics of an assay? (ex: "Analytical Sensitivity, Specificity, Precision, Recovery, Linearity)

The **Analytical Sensitivity** represents the lowest concentration of an analyte that can be statistically distinguished from the background. It is calculated as 2 Standard Deviations above a mean absorbance reading of 20 x Zero standard points.

The **Specificity** is linked to the Cross-reactivity observed when a molecule, other than the analyte of interest, binds to the antibodies in the immunoassay, resulting in a false positive or negative result. Specificity is the degree to which the results are not influenced by cross-reacting substances.

The **Precision** represents how accurately an assay gives the same result for replicates of the same sample.
The intra-assay shows that results are accurate regardless of the sample position within a microplate or a complete rack of tubes (48 tubes).
The inter-assay determines the reproducibility between different runs allowing long term studies.

The **Recovery** represents the ability of the assay to determine the amount of analyte without interferences from the natural components present in that sample matrix.
Recovery is a fundamental check of an immunoassay's ability to measure an analyte accurately.
In a recovery experiment, the final concentration is measured before and after the addition of a known amount of the analyte of interest.

The **Linearity** represents how closely the measured amount of a serially diluted sample matches the expected concentration taking into account the dilution factor.

3.4. How to interpret the Reference values reported in the Instruction for Use?

The values provided in the Instruction for Use are given only for guidance as the reference values can vary according to the population tested and other physiological factors; each laboratory should establish its own normal range of values.

3.5. What is the purpose of the kit controls?

A control material should simulate the physical characteristics and chemical composition of the specimen that is being analyzed. The controls (n = 1, 2 or 3) included in the kit each correspond to a known concentration of the tested analyte. The controls have to be included in the test and allow to validate the test if the values are within the expected range reported on the label. In an assay. the control material is subjected to the same random and systematic errors as the patient's specimen, so it will act as an unknown.

3.6. What are the criteria used to validate a test?

A test can be validated if the concentrations obtained for the kit controls are within the expected ranges reported on the vial labels.
If the results obtained for controls are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practices.

3.7. The calibrators concentrations and O.D readings don't match with the data reported in the Instruction for Use

The Instruction for use generally includes an example of typical calibration curve, for indicative purposes. Those data are for illustration only and should never be used instead of the real time calibration curve.

3.8. Can I use the Calibrators concentrations reported in the Instruction for Use to draw my Calibration curve?

The calibrators/controls concentrations reported on the label of the vials should be used to draw the calibration curve and validate the test. The Calibrators concentrations reported in the Instruction for Use are for illustration only and should never be used instead of the real time calibration curve.

3.9. How to plot a calibration curve?

For ELISA assays:
A bichromatic reading of the microplate can be used; this means that the microplate is read at 450nm. To this values is subtracted its reading at 630 nm as background.
A polychromatic reading follows the same principle but also allows to calculate linearly the Optical Densities that are above 3.0 thanks to a third reading at 490nm filter. The precision is thus ensured for assays whose calibrators' O.D reach high values and can be an advantage for all samples read above 3.0 O.D. This avoids the reader to attribute to the last calibrators a maximum value of 3.0 linked to a “saturation” phenomenon of the reader capacity.

For RIA-IRMA assays:
The user should count the tubes in a gamma counter for 60 seconds. Calculate the mean of duplicate determinations.

Plot the O.D values (ELISA) or B/B0 (%) (RIA-IRMA) (ordinate) for each calibrator against the corresponding concentration (abscissa) and draw a calibration curve, choosing the most appropriate fitting method. Read the concentration for each control and sample by interpolation on the calibration curve.
Computer assisted data reduction will simplify these calculations.

3.10. What fitting calculation should I use to draw my Calibration curve?

If automatic result processing is used, a 4-parameter logistic function is recommended

3.11. What lab equipment is required along with the provided kit box

For Elisa assays, the required material usually corresponds to high quality distilled water, pipettes for delivery of divers volumes (µl), Vortex mixer, Magnetic stirrer, microtiterplate shaker (from 150 rpm to 700 rpm), Washer for microtiterplates, Reader of microplate capable of reading at 450 nm, 490 nm and 650 nm…

For IRMA and RIA assays, the required material usually corresponds to distilled water, pipettes for delivery of diverse volumes (µl), Vortex mixer, tube shaker, automatic syringe for washing, Aspiration system , gamma counter capable of measuring ^{125}I…

3.12. I don't have enough material in the kit for repetitive series, can I mix the material from one kit to another?

It is not recommended to mix the components from different kit lots. DIAsource can guarantee the good performances of a specific validated composition. For that reason, mixed components should be used under the users' own responsibility.

3.13. How can a Certificate of Analysis provided by DIAsource help me ?

For each kit composition tested and sold, DIAsource can provide a certificate of analysis (also called "QC certificate" – "QC report" – "CoA"). That document is kit lot-specific and indicate the lot and expiry date of each reagent included into the box. It also reports the results obtained by our QC Department during the validation of the lot. The Certificate of analysis can help the user to compare his results and guide him in the validation of his test.

3.14. How should I store the products?

The storage recommendations are product-specific (Elisa, IRMA, RIA, Antibodies, …). The information can be found on the kit and components label as well as in the corresponding Instructions for Use.

3.15. Does DIAsource participate to External Quality Scheme?

DIAsource participates to a series of different External Quality Schemes.
Among them,: EQAS from Biorad, DEQAS specific for our Vitamin D Panel, UK NEQAS, Probioqual, ProgBa. The results can be shared upon request.

3.16. Does DIAsource offer extraction methods for Newborn's measurement?

Literature reports that very young children may present high levels of steroid interfering substances. In order to eliminate interfering substances, some laboratories may decide to apply an extraction procedure on the newborn samples.

DIAsource has validated an optional extraction protocol for newborns samples in the frame of the 17OH Progesterone RIA kit (#KIP1409). (See below).

B. Extraction of newborn samples (optional) – FOR SERUM SAMPLE

1. Label one glass tube for each sample (**do not extract calibrators or controls).**
2. Pipette 75 µl of serum, followed by 2.25 ml of diethyl ether.
3. Vortex all the tubes vigorously (2 x 1 minute)
4. Let stand on the table for 5 minutes
5. Place the tubes at -20°C in order to freeze the aqueous phase.
6. Prepare a second series of glass tubes and, for each sample, transfer the organic phase into these new tubes. Avoid contamination by the aqueous phase.
7. Evaporate the organic (ether) phase completely under a stream of air or by placing the tubes at 37°C (water bath). Manipulate under a hood.
8. Dissolve the dry ether extract with 150 µl of Reconstitution solution (**not provided, see VI.)**. Vortex vigorously for 1 minute.
9. Let stand for 10-15 minutes and vortex again for 1 minute. These volumes allow to perform the determination in duplicate.

DIAsource has also validated an optional extraction protocol for newborns samples in the frame of the 17OH Progesterone Elisa kit (#KAP1401). (See below).

B. Extraction for NEWBORN SERUM SAMPLES (optional)

1. Label one glass tube for each sample (do not extract calibrators or controls).
2. Pipette 100 µl of serum, followed by 1.5 ml of diethyl ether(analytical grade; purity > 98%) .
3. Vortex all the tubes vigorously (2 x 1 minute)
4. Let stand the tubes for 15 minutes to separate well aqueous phase (lower phase) and organic phase (upper phase).
5. Then place the tubes at -20°C in order to freeze the aqueous phase.
6. Prepare a second series of glass tubes and, for each sample, transfer the organic phase (upper phase) into these new tubes. Avoid contamination by the aqueous phase.
7. Evaporate the organic phase (diethyl ether) completely under a stream of air by placing the tubes at 37°C (water bath). Manipulate under a hood.
8. Dissolve the dry organic extract with 100 µl of Reconstitution solution (not provided, see VI.). Vortex vigorously for 1 minute.
9. Let stand for 10-15 minutes and vortex again for 1 minute. These volumes allow to perform the determination in duplicate.

3.17. What is a matrix effect?

A matrix effect is a consistent bias in the determination of an analyte between two sources of matrix, such as serum and plasma, which results in erroneous sample readings. The most important type of matrix effect occurs principally when the matrix used to prepare the calibration curve differs from the matrix of the test samples (e.g: test a cell culture supernatant samples (low protein level concentration) while the matrix of the calibration curve is designed to be used with serum sample (high protein level concentration)). A simple dilution of the samples reduces the matrix effect. When diluting the samples, use the same diluent as used for standard curve.

In case you would like to test a sample matrix not listed in the Instruction for Use, please contact our Technical Support Department at the address products.support@diasource.be who will provide you relevant advices.

3.18. Can the DIAsource assays be used for veterinary application?

The DIAsource immunoassays have been developed for human purpose only. For that reason, DIAsource can't be held responsible for data generated on animals samples as a matrix effect could occur.

In regards to frequent customers' feed-back, DIAsource ImmunoAssays maintains a file of published reports and personal communications relative to the application of DIAsource kits for veterinary and animal science research. We took this opportunity to provide freely the information that we receive and we encourage our customers to keep us informed about their testing of DIAsource assays with animal samples.

We created a Species Specific Application catalogue for veterinary application. The cross-reaction information provided in this booklet has not been verified or tested by DIAsource ImmunoAssays but will be an useful support. Our Technical support Department remains at your disposal for further information at the address products.support@diasource.be. Feel open to share with us your own experience results with our assays on animal models.

3.19. Can I use the DIAsource kits on different samples types? (serum, plasma, urine, saliva, CSF…)

It is recommended to use the samples type that has been validated at DIAsource and that is indicated in the Instruction for Use.
In case you would like to test a sample matrix not listed in the Instruction for use, please contact our Technical support Department at the address products.support@diasource.be who will provide you relevant advices.

3.20. Does DIAsource offer an Antibody line?

The DIAsource Monoclonal Antibodies are produced in-house and purified using cutting-edge technologies. The use of these antibodies in DIAsource RIA and ELISA immunoassays for more than 30 years guarantees a high quality and lot-to-lot consistency, and quality control. Our Monoclonal Antibodies are available in the purified unconjugated, purified fragmented and purified biotin conjugate formats.

3.21. Can I use the DIAsource assays on automated systems?

The DIAsource kits have been designed for manual use but many DIAsource assays can be used on automated systems. Upon request, we can provide you Elisa protocols validated on different automates as the GEMINI from Stratec® , as well as RIA protocols validated on the Riamat SR300. We encourage the users to perform a complete validation of a manual kit on their automate as the initial protocol might need few adaptations.
Our Instrumentation Department is continuously working on the validation of new protocols.
Any question relative to instrumentation can be sent to the address instrumentation@diasource.be.

4. BIBLIOGRAPHY

Radioimmunoassay Quality Control and Troubleshooting.
Helen H. Drew