BIVA software 2002

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BIVAconfidence and ***BIVAtolerance*** are two Excel files
for the **B**ioelectrical **I**mpedance **V**ector **A**nalysis
with the **RXc Graph method**

1. *Start* Excel

2. *Menu bar, click* Tools/Macro/Security/Low *(Enable Macros)*

3. *Open* BIVA files

Statistical methods, Software design and validation by Antonio Piccoli
Excel/Visual Basic program by Giordano Pastori

Software requirement: Microsoft® Windows 95 or higher, and Excel 97 or higher.
BIVAconfidence and BIVAtolerance files need < 1 Mb.
The usual disclaimer (i.e. no warranty comes with the codes) and restriction
(i.e. no profit shall be derived from the codes) apply to these computer programs.

Background of BIVA

The conventional approach to the bioelectrical impedance analysis (BIA) uses simple or multiple regression equations to make predictions of masses and volumes of body compartments in subjects with fixed and normal 73% hydration of soft tissues. In abnormal hydration conditions these algorithms can produce biased estimates of body compartments.

Why is BIA based on models and prediction equations ? During stable periods, relations between body components are constant and correlated with each other, which allows investigators to estimate an unknown body component (e.g. TBW, ICW, ECW, FFM, FM, and BCM) from a related measured property (bioimpedance) through regression equations. Hundreds of excellent validation studies established a solid relationship between body impedance and body fluid volume (isotope dilution), but with population specific accuracy of prediction. However, since criterion methods have their own errors, the standard error of the estimate of the best BIA regression equations is too large (95% prediction interval greater than ± 3 to 6 kg or L) to be useful in the clinical setting [1,2].

- ## Patterns vs models and quantitative estimates.

In BIA literature, an **electrical model** (e.g. series, parallel, Cole's, and Hanai's model) is used as an electrical equivalent [3], that is a circuit that electrically behaves like the original, is expressed through mathematical equations, and represents anatomical structures or physical processes (e.g. 75 trillion cells, 3 to 6 body compartments, cellular/vascular fluid shifts, etc).

According to dictionary definition, a **model** is "A representation of the supposed structure of something", and a **pattern** is "A set of forms used as a guide in making things".

In the clinical setting, operational patterns based on direct laboratory data are more useful than complex, explanatory or descriptive models of phenomena.

Both electrocardiography and BIA aim to transform electrical properties of tissues into clinical information. The electrocardiogram (ECG) is a graphic recording through surface electrodes (as in BIA) of electric potentials generated by the heart. Abnormalities of individual waveforms are defined with respect to reference values of healthy subjects. An ECG is interpreted using diagnostic ECG patterns which are a combination of waveforms that was associated with specific cardiac disorders in clinical validation studies (e.g. bundle branch blocks, myocardial ischemia, infarction, etc.) [4]. Calculations of heart volume, ischemic mass volume, infarction volume, etc., from ECG waveforms through electrical models and prediction equations are not used in the clinical setting.

Clinical utility of BIA can be achieved following the methodology of ECG interpretation, that is using **vector BIA** (Bioelectrical Impedance Vector Analysis, **BIVA) as a stand-alone procedure based on patterns of direct impedance measurements (impedance vectors)** [5]. **Body soft tissues** (i.e. lean plus fat soft tissues, or the FFM without bone plus the FM, according to [2]) **actually generate the body impedance** [1-3,6,7] and therefore can be directly evaluated with vector BIA. Contribution of bone to impedance is negligible, and lean contributes more than fat soft tissue because adipocyte droplets of triacylglycerols are non-conductors [1-3,6,7].

- ## Definitions of impedance components [1-3,6,7].

Impedance (Z vector) is a combination of **Resistance (R)** and **Reactance (Xc)** across ionic solutions of soft tissues, tissue interfaces and cell membranes. Impedance at 50 kHz is represented with a complex number (a point) in the real-imaginary plane (**Z vector**), that is a combination of **R** (i.e. the opposition to flow of an alternating current through intra- and extracellular ionic solutions, representing the real part of Z) and **Xc** (i.e. the capacitative component of tissue interfaces, and cell membranes and organelles, representing the imaginary part of Z). The volume of intra and extracellular ionic solutions is (inversely) related to the R component of Z. The amount of soft tissue structures containing the solutions is (directly) related to the Xc component of Z. The arc tangent (Xc/R) is called the **phase angle** (Xc on the ordinate and R on the abscissa axis). Tissue impedance

decreases (R decreases, and Xc first increases, then decreases) with increasing frequency of the stimulating current (1 to 1000 kHz in biomedical analyzers). The **characteristic frequency** of a tissue is the frequency at which the highest Xc values (capacitative component) are obtained, that is the frequency at which the contribution of dielectric properties of soft tissues is at a maximum.

- **Analyzers, electric models, and current frequency.**

In BIA literature, models and prediction equations are used without cross-validation of analyzers and are still preferred to operational patterns. Indeed, prediction equations derived with an analyzer are used with other analyzers while no comparative table with measurement characteristics of analyzers is available from manufacturers.

The National Institute of Health in 1994 [8] and an independent panel of BIA experts in 1997 [9] continued to recommend (in vain) to manufacturers to provide both measured data (R, Xc, |Z|, and phase angle) and prediction equations of their softwares.

The expert panel recommended the utilization of multifrequency BIA (i.e. R extrapolated to zero and infinite frequency) in estimating total body water (TBW from $R_{infinite}$), extracellular (ECW from R_{zero}) and intracellular water (ICW= TBW-ECW) also in altered fluid distribution, and a limited use of single-frequency BIA, parallel model, in estimating body cell mass (BCM). Estimation of fat-free mass (FFM) was considered acceptable only with normal fluid status, and derived estimation of fat mass (FM) was considered inappropriate.

Single-frequency BIA, series model, was ranked as the least useful BIA technique [9].

In 2000, Grimnes and Martinsen reported in their book [3] an extensive update of bioimpedance models and techniques making inconsistent previous recommendations of the expert panels. Relevant discrepancies can be summarized in the following statements:

1) Electric circuit models do not take into account the difference between an electronic and an ionic medium, where biological charge carriers cause relaxation processes, and hence frequency-dependent capacitance and conductance values.

2) When constant-amplitude current is applied to tissues and the corresponding voltage is measured, the raw data and the measured variable are impedance, but the same information can be differently presented as impedance (series circuit models) admittance (parallel circuit models), or as immittance (combined term for impedance and admittance).

3) With multifrequency BIA it is impossible to estimate the extracellular electric volume (i.e. ECW) of tissues since an unknown and variable amount of low-frequency current passes through cells (tissue anisotropy), particularly through muscle fibers (parallel direction).

4) In multifrequency BIA, Hanai's and Cole's models (including multiple Cole systems) are only empirical equations that describe impedance changes with current frequency in suspended spherical cells and in several tissues, other than skin, without any correspondence with tissue structure.

5) For any combination of electrode position, both the influence of skin impedance and the path of deep current through tissues are frequency dependent. Hence, in multifrequency BIA, different proportions of tissues and body segments contribute to low and high frequency impedance, which prevents any reliable determination of body fluid distribution.

6) BIA with a single frequency current close to the characteristic frequency (e.g. 50 kHz) provides the best information at a body level, as it maximizes the signal-to-noise ratio (maximal Xc values in the order of 1/10 of R values), and minimizes both frequency dependent errors and variability of electric flow paths.

How can be compared measurements from different analyzers.

As shown below, after transformation of measured vector components R/H and Xc/H into **bivariate Z-scores** (i.e. R/H and Xc/H minus the mean and divided by the standard deviation of R/H and Xc/H calculated in the reference population), the **RXc-score graph** can be used with any analyzer in any population. In practice, this procedure allows utilization of **standard reference intervals** for impedance vectors. Univariate Z-score are utilized with different DXA analyzers of bone density.

- **Descriptive statistics of impedance components**.
     R and Xc components are significantly, positively correlated in humans, at any age, in healthy and disease [10-23], indicating a binding of ionic solutions to containing tissue structures. Hence, BIA patterns must consider combined changes in both R and Xc components.
     Without need of electric circuit models, **Z can be considered as a bivariate random vector** (with the same propereties as either real or complex vector) [24-28] representing a measurable property of soft tissues. Vector normalization by the subject's height (Z/H, Ohm/m) controls for the different conductor length. Impedance is proportional to the conductor length, and stature is a surrogate for the conductor length of human body from hand to foot.
     The **intersubject variability of Z** is appropriately represented with the bell-shaped bivariate normal distribution, that is with (positive) sloping, elliptical probability regions on the R-Xc plane, which can be represented with probability graphs like the **RXc graph**, that is with curves or surfaces showing the values of a probability function for the joint distribution of R and Xc values.
     Bivariate Z-scores: after transformation of measured vector components of the RXc graph into **bivariate Z-scores** [25-28] (i.e. R/H and Xc/H minus the mean and divided by the standard deviation of R/H and Xc/H calculated in the reference population), the **RXc-score graph** [23] can be used with any analyzer in any population through its **standard reference intervals**.

Statistical inference with the RXc graph method

     The two components of the impedance vector Z, namely R, and Xc, standardized by height (H), are considered as two correlated normal random variables (i.e. a bivariate gaussian vector) forming elliptical probability regions on the RXc plane, which are **confidence ellipses for mean vectors** and **tolerance ellipses for individual vectors** [10,12,16,23-32].

- **Tolerance ellipses for individual impedance vectors.**
     The bivariate reference intervals of the healthy population are tolerance ellipses, which are also known in literature as prediction ellipses, or isodensity probability ellipses, or confidence ellipses for an observation.
     Three tolerance ellipses are considered in the RXc graph, namely the median, the third quartile, and the 95th percentile, that are regions including 50%, 75% and 95% of individual points, respectively. These three tolerance ellipses allow a more detailed classification of vector position than the two (75% and 95%) that were used in the first article presenting the RXc graph method [10].
     By plotting the two components R/H and Xc/H measured in an individual subject as an individual impedance vector (a point) on the RXc graph, one can directly rank its distance from the reference mean vector through the tolerance ellipses (RXc point graph). Below, in the figure of BIVA patterns, a seven-point scale is obtained considering upper and lower halves of tolerance ellipses larger than 50%.
     The distance of the broken line connecting point vectors of repeated measurements in a same subject can also be ranked and compared with the reference, tolerance ellipses (RXc path graph).

- **Confidence ellipses for mean vectors**.
     By plotting the two mean components R/H and Xc/H measured in a group of subjects as a mean impedance vector with its 95% confidence ellipse, one can directly establish the mean vector position and variability in the corresponding population. The sample mean is presented as estimate of results that would be obtained if the total population were studied. The lack of precision of a sample mean which results from both the degree of variability in the factor being investigated and the limited size of the study, can be shown advantageously by a confidence interval.

- **Comparison of mean vectors through confidence ellipses** [25,29-331.
     As with univariate analysis of continuous, normal variables, the confidence intervals show where means are and can be utilized to test whether a mean is significantly different from some hypothesized value .

The inspection of a series of confidence intervals is very useful from a descriptive point of view and can help in interpreting statistical significance of tests (i.e. appreciating clinical importance of "significant" differences). It is also the simplest way to rank the **Mahalanobis' generalized distance (D)** among mean vectors, which uses within-groups variation (elliptical shape) as a yardstick for differences between means (e.g. if D= 4 between two vectors, then vectors differ by 4 within-groups variation). Multivariate statistical tests utilize functions of D.

As statistical tests of significance are related to confidence intervals of means, one can use confidence intervals for approximate comparison of vector position of different populations.

In general, with unbalanced groups, if the 95% confidence intervals of group means do not overlap, the group means are significantly (P < 0.05) different, but the reverse is not necessarily true. Indeed, there are situations where confidence intervals overlap only slightly, but the statistical test still declares a significant difference at the 5% level. This is due to the way of pooling group variances (assumed to be equal) in statistical tests and to the lower probability that two events occur jointly instead of separately. In comparison of means from two groups with a same sample size and a same variance, the graphic criterion using confidence ellipses is equivalent to the statistical test.

Hence, the graphic criterion based on confidence intervals is more conservative than statistical tests as it tends to declare not significant small differences between mean values with overlapping confidence intervals (meaning that mean vectors are very close and the statistical difference is not important).

However, the most efficient statistical approach for comparing two sample means is based on their difference both in statistical tests and in graphic procedures. If the 95% confidence ellipse of mean vector difference does not cover the zero point, then the statistical test is significant at P< 0.05. If it covers the zero point, the statistical test is not significant.

With *BIVAconfidence* file one can plot up to 128 mean vectors with their 95% confidence ellipse and perform the two-sample Hotelling's T^2 test (*Mean graph* sheet).

One can also plot up to 128 mean difference vectors with their 95% confidence ellipse and perform the paired one-sample Hotelling's T^2 test (*Paired graph* sheet). If a 95% confidence ellipse of a vector displacement covers the null vector (0,0), then the vector displacement is not statistically significant (P< 0.05) at a paired one-sample Hotelling's T^2 test. The full equivalence between statistical test and graphic critrerion is due to the use of confidence intervals of the difference in paired analysis.

Mahalanobis' D is provided in either procedure (D between two mean vectors, and D from zero, respectively).

- **Statistical tests on multivariate mean vectors** [24-29,34].

The **two-sample Hotelling's T^2 test** is just a multivariate extension of the Student's t test for unpaired data in comparison of mean vectors (i.e. two or more variables) from two groups. It is more sensitive than the Student's t test performed on each variable and entails a smaller risk of erroneously rejecting the null hypothesis.

The **paired one-sample Hotelling's T^2 test** is a multivariate extension of the Student's t test for paired data in comparison of mean difference vectors (i.e. the differences of two or more variables considered two times in the same subjects).

Both the two-sample and paired one-sample Hotelling's T^2 tests are available in the *BIVAconfidence* **file (***Mean graph***, and** *Paired graph* **sheet, respectively).**

These tests are reasonably robust against departures from normality of data. They are also available in the BMDP statistical package.

Multivariate analysis of variance (MANOVA) is available in statistical softwares for comparisons of either mean vectors or mean difference vectors from more than two groups. Comparisons are made through multivariate tests like Wilks' lambda, Hotelling-Lawley trace, Pillai's trace, and Roy's maximum root which are transformed into F statistics for calculation of test probability and are equivalent to the Hotelling's T^2 test in the comparison of two groups (BMDP, SAS, and SPSS softwares). As in univariate ANOVA, only approximate, complex procedures are available for multiple comparisons in MANOVA.

The RXc graph method in clinical bioimpedance analysis [10-23, 35-41].

- **The RXc graph method makes three kinds of bioimpedance evaluation possible**:

    1) **Evaluation of a single vector** measured the first time in an individual subject, plotting the point vector on the reference bivariate tolerance ellipses *(RXc point graph)*.

    2) **Evaluation of patient's bioimpedance follow-up**, plotting on the reference tolerance ellipses the broken line of random trajectory depicted by the successive measurements of the impedance vector in an individual patient *(RXc path graph)*.

    3) **Evaluation of groups of subjects** using the bivariate **95% confidence ellipses of the mean vectors** (*RXc mean graph).* This is useful for clinical research studies aimed to identify disorders in body composition.

- **Interpretation of individual vector position on the RXc graph**

    Vector BIA with the RXc graph method, allows an evaluation of soft tissues through patterns based on percentiles of their electrical properties without prior knowledge of body weight.

    From clinical validation studies in adults, vectors falling out of the 75% tolerance ellipse indicate an abnormal tissue impedance, which is interpreted and ranked following the two directions of major and minor axis of tolerance ellipses:

    1. Vector displacements parallel to the major axis of tolerance ellipses indicate progressive changes in tissue hydration (dehydration with long vectors, out of the upper pole, and hyperhydration with apparent edema with short vectors, out of the lower pole);

    2. Vectors falling (steady state) or migrating (dynamic state) parallel to the minor axis, above (left) or below (right) the major axis of tolerance ellipses indicate more or less cell mass, respectively, contained in soft tissues (i.e. vectors with a comparable R value and a higher or lower Xc value, respectively).

    3. Different trajectories indicate combined changes in both hydration and tissue mass.

BIVA patterns
Major axis => tissue hydration, minor axis => soft tissue mass
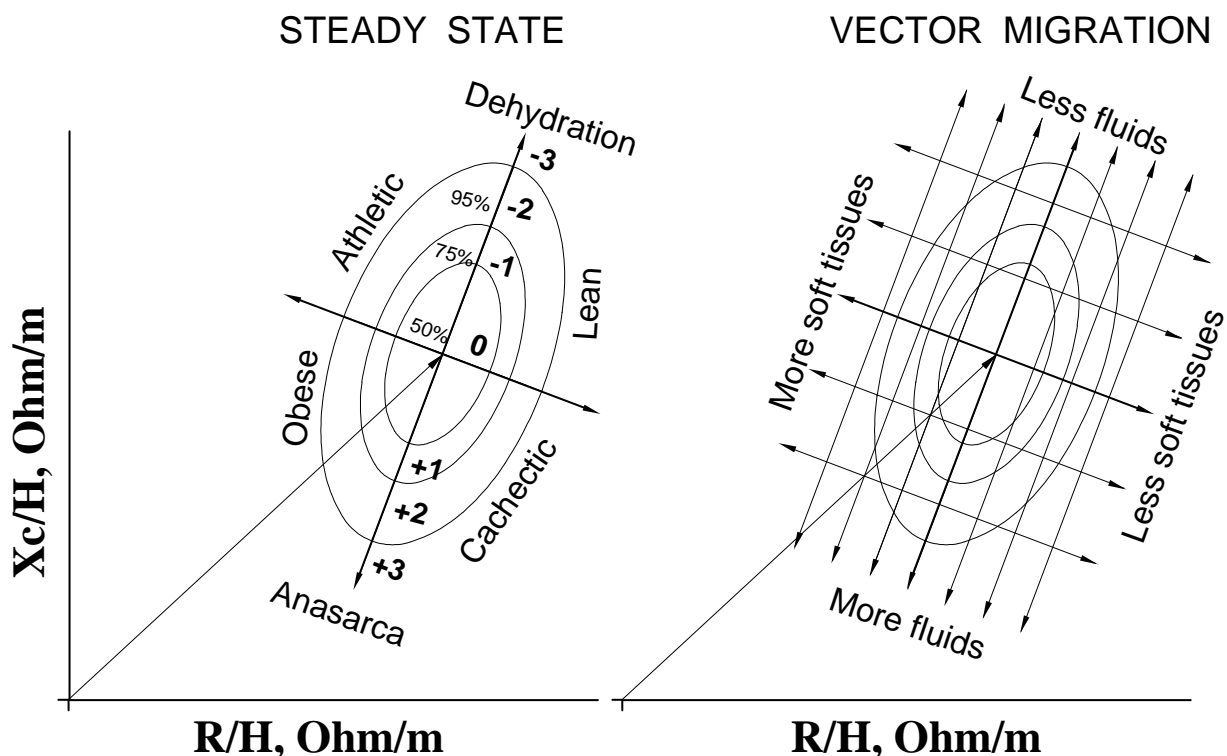
- **BIVA patterns from literature through the RXc-score graph**

Data in the figure are drawn from literature and plotted on the RXc-score graph after transformation of impedance measurements from several disease groups into bivariate Z-scores (with respect to their reference population) (details and literature in reference 23).

Solid and open circles represent male and female gender, respectively.

Single score vectors are from athletes, obese subjects of class I to III (Ob/1-3) or class I (Ob/1) [NHANES III population], patients with chronic renal failure (CRF) in conservative treatment, nephrotic syndrome (edema), lung cancer, AIDS in stage WR 3-5 (HIV/3-5) or WR 6 (HIV/6), and anorexia nervosa.

Repeated score vectors are from climbers before and after high altitude dehydration, hemodialysis (HD) patients, either lean or obese (HDo) before and after fluid removal with a dialysis session, and dehydrated patients with cholera before and after fluid infusion.

A forward or backward displacement of vectors parallel to the major axis of ellipses was associated with dehydration or fluid overloading, respectively, reaching extremes out of the poles. Vectors above or below the major axis (meaning upper-left or lower-right half of ellipses) were associated with more or less cell mass in soft tissues, respectively, with extremes along the minor axis.
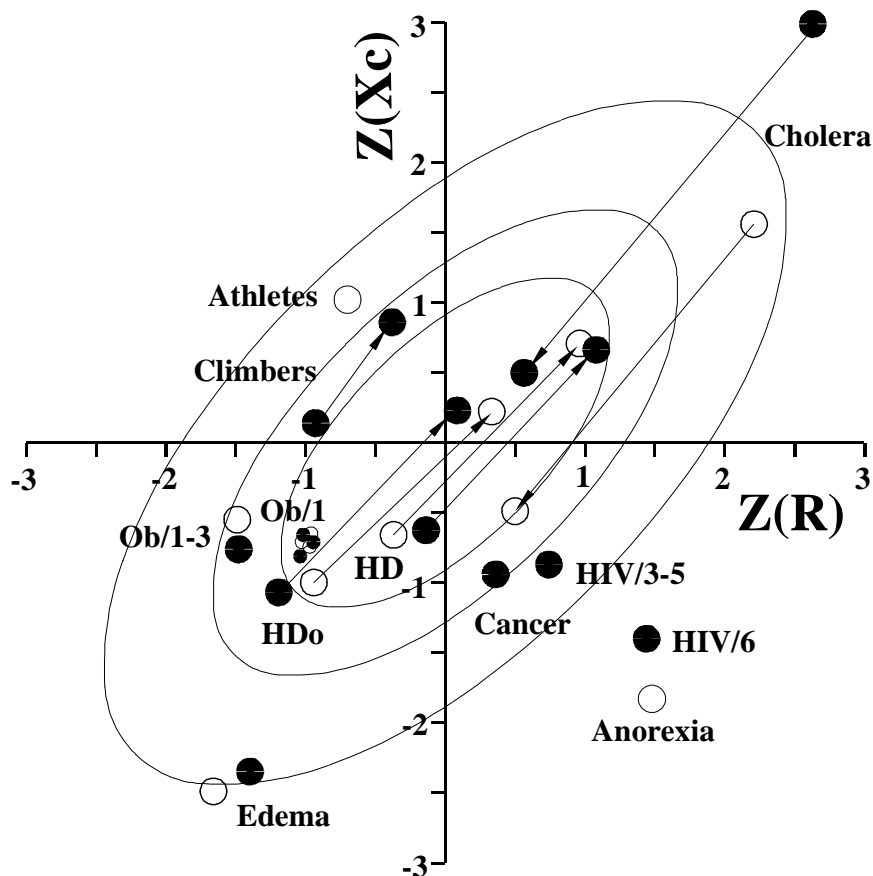
The BIVA software

BIVAtolerance file

Concepts

In the clinical setting, one is more concerned about where individual measurements might fall on the R-Xc plane, with respect to the tolerance ellipses of the reference population**.**

Three tolerance ellipses are drawn corresponding to the 50th, 75th and 95th percentile of the impedance vector distribution of a selected, reference population.

Options for vector analysis with *BIVAtolerance*:

1. Plot of one point vector for every subject on the tolerance ellipses (**Point graph** sheet)

2. Plot of one bivariate Z-score vector for every subject on the reference **RXc-score graph** with three tolerance ellipses (**Z-graph** sheet)

3. Plot of a sequence of point vectors for one subject on the tolerance ellipses (**Path** sheet)

Opening the BIVAtolerance file

- As this Excel file uses Visual Basic macros, a dialog box appears asking whether you agree to **start macros,** which are potential targets for virus (like other .exe files).
  Click on the agreement option.

- The workbook of the *BIVAtolerance* file has 7 sheets:
    Guide (a summary of this text)
    Reference populations
    Point graph
    Path
    Subjects
    Z-score
    Z-graph

Reference populations sheet

- It is a database with mean impedance vectors of populations from literature or from your studies. *The program only reads the first row (yellow).*

- **One row** contains statistical parameters of **one population**.

- **The program uses A to I columns.** Columns from J on are free for the user.

- **Don't change the order of A to I columns.**

- Row cells from A to I are: Population identification Code, Population size (N), Mean and Standard deviation (SD) of both R/H and Xc/H, with their linear correlation coefficient (r), Sex (M,F or m,f), and a text free legend with population characteristics.

- **The selected, reference population must be in the first row** (yellow row).
   Hint: select one row, copy and paste that row on the first row of the worksheet.

- **Append more rows in the worksheet for additional, reference populations, with a different Popul(ation) Code** (column A) .

Point graph sheet

- The 50%, 75%, and 95% tolerance ellipses are drawn for **your selected, reference population** (i.e. the population in the first, yellow row at the top of the *Reference populations* sheet).

- The dialog box **Select groups** allows selection of up to 10 classification groups of subjects (codes are recorded in the *Group code* column in the *Subjects* sheet) whose impedance vectors will be plotted as points on the RXc point graph with different symbols by group.
  Exit the dialog box without action: click on the **X** *botton.*

- **One vector is plotted for each valid row in the *Subjects* sheet** (35,500 rows in Excel 97, or more). The program only draws **points of subjects who meet 4 conditions in their row:**
  A numerical value in the *Subject ID* cell (column A)
  The value = 1 in the *Seq(uence)* cell (column B)
  A value > 0 in the *Popul(ation) code* cell (column J)
  A value > 0 in the *Group code* cell (column K)
  To skip rows: leave blank in the *Group code* cell (column K)

- **Change graph characteristics** (color and size of lines and symbols, axis scale, legend text, dimension and position of the graph) using the double click on graph entities (Excel rules).
  Return to the default graph size with a click on the upper menu bar botton **RESET GRAPH**.

- **Plot different group(s)** with a click on the upper menu bar botton **NEW GRAPH**.

Path sheet

- The 50%, 75%, and 95% tolerance ellipses are drawn for **your selected, reference population**.

- The dialog box **Select person** allows selection of one subject by surname (i.e. one row in the *Subjects* sheet). *Exit the dialog box without action: click the* **X** *botton.*

- The RXc path graph will start with **an arrow from the origin to the first point vector of the sequence** (i.e. the row in the *Subjects* sheet with the value 1 in the *Seq(uence)* cell (column B)) and will continue plotting other vectors that are read in the subsequent rows with a same *Subject ID*.

- **Only the order of vectors** (along a broken line), not the time interval between vectors is considered (date is not considered). **For one subject, up to 35,500 vectors are plotted** (Excel 97).

- **The program draws points of one subject whose rows have a positive number in the *Subject ID* cell** (column A)**, and the value = 1 in the *Seq(uence)* cell** (column B, the first point of the follow-up path). To skip vectors (rows) following the first one, leave a blank in the *Subject ID* cell.

- Plot a **different subject's path graph** with a click on the upper bar botton **NEW GRAPH**.

Subjects sheet

- **Input columns are A to L.**
  The **reference Population Code of each subject** must be in column J (parameters of the corresponding population are in the *Reference Populations* sheet).
  Columns Q (Date of measure) and R (Date of birth) are just labels for documentation.
  Output columns are M to P. Columns from T on, are free for the user.

- **Do not change the order of columns from A to P.** You can change the content of cells, the format of numbers, and the size of columns, with Excel tools. Following changes in input columns, update output columns with a click in the upper menu bar **CALCULATE**

- *Hint for importing data from other Excel or Excel-compatible files*: copy by column from a different file and past by column on the *Subjects* sheet (A to L columns).

Z-score sheet

- **Input columns for parameters of the reference population are A to F.**
  Input columns for subject's data are G to J. Column K is for Drawing option.
  Output columns are L and M.
  Column N is free for Notes, labels of groups, names and characteristics of subjects.
  Columns from O on, are free for the user.

- Row cells from A to J are: Population identification Code, Population size (N), Mean and Standard deviation (SD) of both R/H and Xc/H, Subject ID code, Group Code, and the individual value of both R/H and Xc/H.
  Hint for importing data in columns A to F (from *Reference populations* sheet, columns A to F) and in columns I to J (from *Subjects sheet*, columns M to N): copy cells by row and past on the corresponding row cells on the *Z-score* sheet.

- **Do not change the order of columns from A to N.** You can change the content of cells, the format of numbers, and the size of columns, with Excel tools. Following changes of cell values in input columns, output columns are updated with a click in the upper menu bar **CALCULATE.**

Z-graph sheet

- **The 50%, 75%, and 95% tolerance ellipses are drawn for the standard, reference RXc-score graph** representing the bivariate distribution of the standard deviates of R/H and Xc/H with **zero mean, unit standard deviation, and their correlation coefficient r = 0.638** (NHANES III estimates on 8022 adults) according to the RXc-score graph methodology [23] (formulas in the Appendix).
  The two standard deviates of the bivariate Z-score vector are calculated from individual R/H and Xc/H data as $Z(R)$= (R/H - the mean value)/ SD, and $Z(Xc)$= (Xc/H - the mean value)/ SD, that is with respect to the means and standard deviations of a specific, reference population by race, gender, BMI range, age range, and analyzer.
  The individual impedance vector deviates are plotted on the **RXc-score graph (dimensionless scale)** allowing classification of a subject into the same categories as with the original impedance vector on the **RXc-graph (Ohm/m scale)**, i.e. falling within the median, 3rd quartile, or 95th percentile, or outside the reference intervals.

- Different symbols by group of subjects are used up to 10 classification groups (codes 1 to 10 are recorded in the *Group code* column in the *Z-score* sheet) whose score vectors are plotted as points on the RXc-score graph.

- **One score vector is plotted for each valid row in the *Z-score* sheet** (35,500 rows in Excel 97, or more). The program only draws **points of subjects who meet 2 conditions in their row:**
  A value > 0 in the *Group code* cell (column H)
  The value = 1 in the *Drawing option* cell (column K)
  To skip rows: leave blank in the *Drawing option* cell (column K)

- **Change graph characteristics** (color and size of lines and symbols, axis scale, legend text, dimension and position of the graph) using the double click on graph entities (Excel rules).
  Return to the default graph size with a click on the upper menu bar botton **RESET GRAPH**.

- The upper menu bar botton **NEW GRAPH** remind you to change the value in the *Drawing option* cell in the *Z-score* sheet to plot different subjects.

The BIVA software

BIVAconfidence file

Concepts

Unpaired data analysis. In clinical research, one is mainly interested in point (bivariate mean vector) and confidence interval (ellipse) estimation of mean vectors and in comparing mean vectors from different groups of subjects. **The 95% confidence ellipses are drawn for the mean impedance vectors of 1 to 128 groups.**

Paired data analysis. Mean vectors can also be **mean vector displacements** which are differences (e.g. post minus pre values, or pre minus post values) of both vector components.

Options for vector analysis with *BIVAconfidence*:

1. **Plot of the mean vector with the 95% confidence ellipse for every group of subjects** (*Mean graph* sheet).
   Separate 95% confidence ellipses are equivalent to a statistically significant (P< 0.05) **two-sample Hotelling's T^2 test** (*Mean graph* sheet).

2. **Plot of the mean difference vector (vector displacement) with the 95% confidence ellipse of the difference for every group of subjects.**
   If a 95% confidence ellipse of a vector displacement does not cover the origin of the dRXc graph, then the vector displacement is statistically significant (P< 0.05), which is equivalent to a significant **paired one-sample Hotelling's T^2 test** (*Paired graph* sheet).

3. The *Mean graph* sheet can also be used to draw 95% confidence ellipses of **vector displacements of different groups** of subjects, recorded as differences in the *Groups* sheet.
   This exploits the property that separate 95% confidence ellipses are equivalent to a statistically significant (P< 0.05) two-sample Hotelling's T^2 test (mean differences from independent groups).

Opening the BIVAconfidence file

- As this Excel file uses Visual Basic macros, a dialog box appears asking whether you agree to **start macros,** which are potential targets for virus (like other .exe files).
  Click on the agreement option.

- If you open *BIVAconfidence* file when *BIVAtolerance* file is also open, you will see the additional commands of both files on the Excel's upper menu bar.

- The workbook of the opened file has 5 sheets:
    - Guide (a summary of this text)
    - Groups
    - Mean graph
    - Paired data
    - Paired graph

Groups sheet

- It is a database with mean impedance vectors of groups of subjects from literature or from your studies. **One row** contains statistical parameters of **one group**.
- **The program uses A to I columns.** Columns from J on are free for the user.
- **Don't change the order of A to I columns.**
- **Row cells from A to I** are: Group IDentification code, Group sample size (N), Mean and Standard deviation (SD) of both R/H and Xc/H, with their linear correlation coefficient (r), Drawing option code, and a text free legend with group characteristics.
  Hint: in columns from J on, put means and SD of measured impedance and anthropometric variables, like R, Xc, Height, Weight, BMI, and other data, like age, and organ function indexes, that are useful for group description in articles.
- **The mean value and SD of R/H and Xc/H** (columns C to F) **must be calculated on individual subject's data** (in a different sheet). However, if the range of Height is narrow (e.g. adults by gender and race), they are close to to the ratio of mean value and SD of R and Xc divided by the mean value of the Height.
- **The correlation coefficient between vector components must be calculated on individual subject's data.** However, if the range of Height is narrow, then r(R/H, Xc/H) is close to r(R, Xc) value.
- Insert rows or append more rows in the worksheet for additional groups, with a different Group ID code (column A) .

Mean graph sheet

- **The 95% confidence ellipses are drawn for all groups whose *Drawing option* cell** (column H) in the *Groups* sheet, **contains either the value 1** (draws an ellipse with a dot indicating the mean vector) **or 2** (draws an ellipse with an arrow from the origin, indicating the mean vector).
  Ellipses of groups with a blank in the *Drawing option* cell are not plotted.
- **One vector can be plotted for each valid row in the *Groups* sheet (up to 128 ellipses per graph).**
- **Change graph characteristics** (colors, size, axis scale, legend text, dimension, and position of the graph) using the double click on graph entities (Excel rules). Default scale intervals of axes are appropriate for adults' vectors. **Adapt the scale** for long vectors (e.g. children's vectors) using Excel tools.
- **Return to the default graph size** with a click on the upper menu bar botton **RESET GRAPH**.
- **Two mean vectors,** from two independent groups of subjects, **can be compared with the two-sample Hotelling's T^2 test**. Click on the upper menu bar botton **TEST**.
  A **dialog box** shows a table in the left containing the *Group ID codes* of plotted ellipses. The highlighted group code changes to red the colour of the corresponding ellipse. **Two groups can be moved to the right boxes for test with a click on the arrow bottons.**
  Click the lower right button **TEST** to show test statistics: Hotelling's T^2 test value with the corresponding **F** test and **P** values, and the Mahalanobis' generalized distance **D**.

Paired data sheet

- It is a database with **mean difference of two impedance vectors measured in two conditions in a same group of subjects** (e.g. post- minus pre-hemodialysis impedance vector). **Coordinates of the individual difference vector (dZ/H, or vector displacement) are the differences in both R/H (dR/H) and Xc/H (dXc/H), which are correlated each other.**
  The mean difference vector can fall in any of the four quadrants of the dR-dXc plane.
  One row contains statistical parameters of impedance vector displacement of **one group**.

- **The program uses A to I columns.** Columns from J on are free for the user.

- **Don't change the order of A to I columns.**

- Row cells from A to I are: Group IDentification code, Group sample size (N), Mean and Standard deviation (SD) of both dR/H and dXc/H, with their linear correlation coefficient (r), Drawing option code, and a text free legend with group characteristics.

- **The mean value and SD of dR/H and dXc/H** (columns C to F) **must be calculated on individual subject's data** (in a different sheet).

- **The correlation coefficient between vector component differences, r(dR/H, dXc/H), must be calculated on individual subject's data**(in a different sheet).

- Insert rows or append more rows in the worksheet for additional groups, with a different *Group ID code* (column A) .

Paired graph sheet

- **The 95% confidence ellipses are drawn for all groups of differences whose *Drawing option* cell**(column H) in the *Paired data* sheet, **contains either the value 1** (draws an ellipse with a dot indicating the mean vector) **or 2** (draws an ellipse with an arrow from the origin, indicating the mean vector).
  Ellipses of groups with a blank in the *Drawing option* **cell are not plotted.**

- **One vector can be plotted for each valid row in the *Paired data* sheet (up to 128 ellipses per graph).**

- **Change graph characteristics** (colors, size, axis scale, legend text, dimension, and position of the graph) using the double click on graph entities, according to usual Excel rules.
  Default scale intervals of axes are appropriate for most vector displacements in adults.
  Adapt the scale for large vector displacements using Excel tools.
  Return to the default graph size with a click on the upper menu bar botton **RESET GRAPH**.

- **A mean vector displacement in one group of subjects can be evaluated with the paired one-sample Hotelling's T^2 test**. It is a test of the null hypothesis (Ho) that no vector displacement was observed in the population in the two conditions (i.e. that the vector of the mean difference is dZ/H= (0, 0), or at the origin of the RXc graph).
  The test can be perfomed with a click on the upper menu bar botton **TEST**.
  A **dialog box** shows a table containing the *Group ID codes* of plotted ellipses with test statistics: paired one-sample Hotelling's T^2 test value with the corresponding **F** test and **P** values, and the Mahalanobis' generalized distance **D** from zero.

FORMULAS FOR CALCULATION OF CONFIDENCE AND TOLERANCE ELLIPSES

Geometrical parameters for drawing the RXc Graph and the RXc-score Graph

In the case of bivariate normal distribution, confidence and tolerance intervals can be calculated by exact methods [24-28].

After suitable modification of formulas, common statistics of the simple linear correlation analysis can be used for calculation [10,23].

Given n pairs of observations x and y, with standard deviation s_x and s_y, and correlation coefficient r, for a fixed α probability level, take the Snedecor's F_α value with 2 and n-2 degrees of freedom.

RXc graph.

The **semi-axes L_1 and L_2** and the **slopes b_1 and $b_2 = -1/b_1$**, of **the axes of the 100(1-α)% confidence and tolerance ellipses** (e.g. α= 0.05, 0.25, and 0.50 for the 95th, 75th, and 50th percentile, respectively) of RXc graphs can be calculated using equations (1a) and (2a), respectively.

RXc-score graph.

Parameters of **tolerance ellipses** of bivariate Z-scores (RXc-score graph) can be calculated accordingly, using equations (1b) and (2b) [23].

$$L_1, L_2 = \sqrt{K} \cdot \sqrt{(n-1)(s_x^2 + s_y^2) \pm \sqrt{[(n-1)(s_x^2 + s_y^2)]^2 - 4(n-1)^2(1-r^2)\,s_x^2\,s_y^2}} \qquad (Eq.\ 1a)$$

$$L_1, L_2 = \sqrt{K} \cdot \sqrt{2(n-1) \pm 2r(n-1)} \qquad (Eq.\ 1b)$$

where
$K = F/\,n\cdot(n-2)$ for confidence ellipses
$K = F\cdot(n+1)/\,n\cdot(n-2)$ for tolerance ellipses

$$b_1, b_2 = (b, -1/b) = (s_y^2 - s_x^2)/\,2\,r\,s_x\,s_y \pm \sqrt{1 + [(s_y^2 - s_x^2)/\,2\,r\,s_x\,s_y]^2} \qquad (Eq.\ 2a)$$
$$b_1, b_2 = \pm 1 \qquad (Eq.\ 2b)$$

Hotelling's T^2 test, and Mahalanobis' generalized distance D

A simple presentation of both the two-sample, and paired one-sample Hotelling's T^2 test, as well as of the Mahalanobis' generalized distance D can be found in reference 29, on pages 464-479.

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Exporting graphs

- As this Excel file uses Visual Basic macros, a dialog box appears asking whether you agree to **start macros,** which are potential targets for virus (like other .exe files).

  Click on the agreement option.

Hint for preparing PowerPoint slides

1. **Copy** a graph from a *BIVA software* sheet
2. **Paste** in a new PowerPoint slide (open either a *layout with title* or *empty layout)*
3. Make any change you like, e.g. change size, add text labels, arrows, lines, colours, etc.

Hint for preparing figures in text documents, Word files

- *Copy/Paste directly from Excel BIVA sheets to Word*
1. **Copy** a graph from a *BIVA software* sheet
2. **Paste** in the selected line of page
3. Adapt graph size, add text labels, etc.

      or

- *Import from PowerPoint to Word*
1. Prepare a PowerPoint slide, as above
2. **Insert** (upper menu bar of Word) the PowerPoint slide in the selected line of the document as an **Object** (created as a PowerPoint file)

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