

# **(Human bLOod Range vAlidator)**

## **HORA v1.1**

### **TUTORIAL**

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## Introduction

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HORA suite (Human bLOod Range vAlidator) is a Java application developed to validate the metabolomic analysis of human blood. It bases the search work on a database, built in our laboratory, that stores the normal plasma and serum range concentrations of metabolites. The application is available for Windows 2000/NT and WP platform.

The goals of HORA are:

- to find, given a list of metabolites, which ones are outside of the normal range;
- to show the metabolites that are not present in the list provided by the user;
- to supply a graphical interface to manage the data of the database

## Installation

HORA suite is available on the PaternostroLab website in the “downloads” section:

[www.paternostrolab.org](http://www.paternostrolab.org)

The HORA\_v1.1.rar contains two folders:

- **HORA**: this folder contains the Graphic interface of HORA, with:
  - ↳ srs: file source folder
  - ↳ bin: file compiled folder
  - ↳ var: variable (ex: icon) folder
  - ↳ mysqlconnector.jar
- **HORA\_DB**: this folder contains the database of metabolites relative the human blood

Unzip the file and follow the next instructions.

### **Database (HORA\_DB folder inside HORA\_v1\_1.rar)**

The HORA database is built in MySQL 5.0., you can download it from:

<http://dev.mysql.com/downloads/mysql/5.0.html#downloads>

Here you can choose the appropriate Windows version to download.

Unzip the downloaded file and start the installation. Here you can find a brief list of the steps to follow during the installation. All the choices are optional, except the last one concerning the setting of user and password for the access to the server.

For HORA you can choose:

- Detail configuration

- Typical installation
- Skip Sign – Up for the MYSQL.com account

For the server configuration installation you can choose:

- Developed Machine
- Multifunctional database
- Decision support (DSS)/OLAP
- Enable TCP/IP networking: port 3306 and enable strict mode
- Standard character set
- Install as windows service and included bin directory in windows path

-  **Security option account: (this in not optional)**

↳ **user** : root

↳ **password**: root

- execute

There are more tools that you can download from this website that can help you manage the MySQL database by yourself, but the advantages of HORA is that it implements a set of embedded procedures to guarantee the consistency of the database.



**In any case it is well-recommended to install the MySQL Administrator 1.2 to manage the MySQL server.** <http://dev.mysql.com/downloads/gui-tools/5.0.html>

HORA database is in the HORA\_DB folder, you have to copy this folder into the data folder of MySQL:

- Open the folder "data" in MYSQL folder, usually located in the system folder "Program File" (ex: C:\Program Files\MySQL\MySQL Server 5.0\data)
- Copy the DATA\_DB folder here;

### **Graphic interface (HORA folder inside HORA\_v1\_1.rar)**



**HORA is a java application and thus needs the installation of the Java Virtual Machine. You can download if from the SUN website. Java Runtime Environment Version 5.0 Update 9) and select typical installation:**

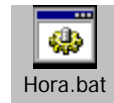
<http://www.java.com/en/download/index.jsp>

The default path of installation of HORA is: C:\bin, but you can change it; in this case you have to follow the next instruction where [mypath] is the new path that you choose.

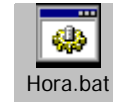
- copy HORA folder in C:\bin (If doesn't exist create this folder in the File System) or in [mypath]



**If the [mypath] is different from c:\bin you have to set the hora.bat file:**



- open hora.bat with an editor like Word or Notepad (right-click the batch file and select 'edit')
- change the variable "horapath" (set horapath=...) with [mypath\_hora] ex: set horapath=C:\Documents and Settings\Joe\Desktop\HORA



Hora's graphic interface is launched by clicking the hora.bat contained in HORA folder.

The HORA database is divided into two different sections:

- Blood section
- Synonyms section

### Blood section

This section contains two tables: blood and bibliography. The blood table contains all the ranges collected from the sources specified in the bibliography table.

### Synonyms section

This section contains two tables: IUPAC and Synonyms.

- IUPAC contains the list of the PLBLD codes with the IUPAC description ([www.iupac.org/dhtml\\_home.html](http://www.iupac.org/dhtml_home.html));
- Synonyms contains the name list of synonyms, each one linked with one or more PLBLD codes.

The association between a name and a PLBLD code must be unique, as must be unique the association between an IUPAC code and a PLBLD code. In the following example you can see that in the database there are five instances of "Adenosine", each with different PLBLD code but each of them is a unique association.

code	name	IUPAC
1161	Adenosine	3H-Imidazo[2,1- <i>i</i> ]purine, 3-(2-deoxy- $\beta$ -D-ery
2170	Adenosine	(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-2-(6-amino-2-chloro-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol
2723	adenosine	6-amino-9-[3,4-dihydroxy-5-[(hydroxy-sulfooxy-phosphoryl)oxymethyl]oxolan-2-yl]-purine
380	Adenosine	2-(6-aminopurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol
4668	adenosine	[[[[5-(6-aminopurin-9-yl)-3,4-dihydroxy-oxolan-2-yl]methoxy-hydroxy-phosphoryl]oxy-hydroxy-ph

### What is PLBLD code

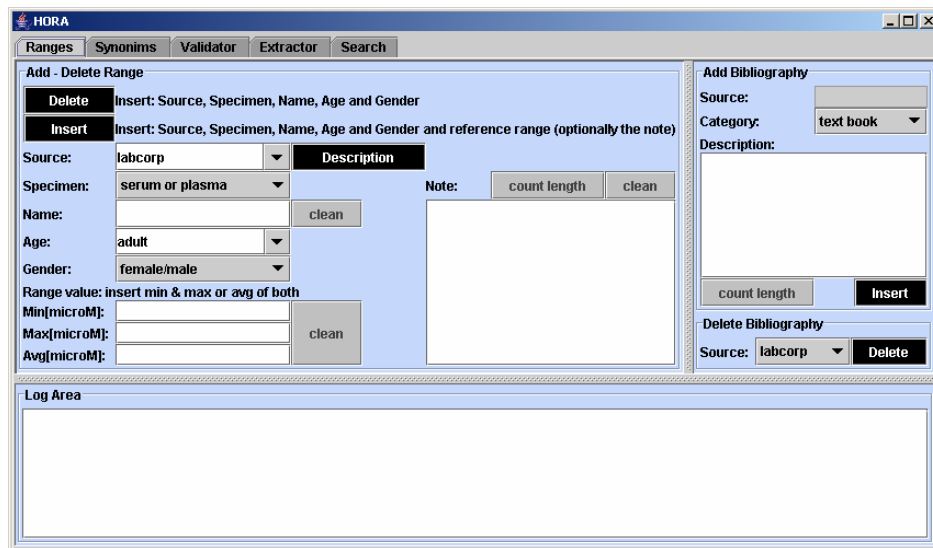
The PLBLD code (PaternostroLab Blood Data) is the HORA code which is unique for all IUPAC codes. It is automatically created by HORA when you insert a new list of Synonyms with their accompanying IUPAC code.

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## HORA - Graphic Interface - Ranges Panel

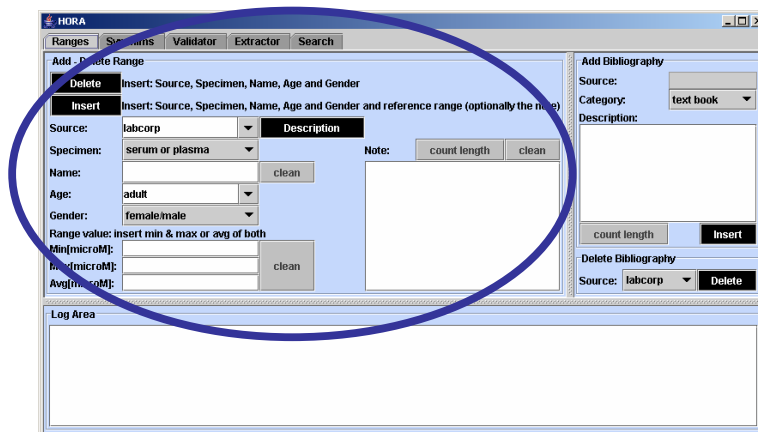
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The Ranges Panel is the first panel that it is opened when you start the HORA application. With this panel you can manage the information contained in the HORA database that relates to the Blood section. For instance, you can insert a new reference range, delete an existing reference range, insert or delete a bibliography entry, or check the description of a bibliography entry.



You can't directly modify the information contained in the database, but you can delete the old information and insert the correct one.

### Reference Range



## Add new reference range

In the panel "Add-Delete Range" you can insert a new Range by using the following steps:

- Select the **source** you wish to use from the list given. (If your source is not present in the list, type its name in the **source** box and when you insert your range you will be able to add it to the database in the "Add Bibliography" panel as described later).
- Set the **specimen**:
  - Serum or plasma
    - ↳ plasma
    - ↳ serum
    - ↳ blood - whole blood;
- Insert **name**



**Name can contain letters, numbers, and symbols, but not quotation marks: ". All names inserted in this part of database must be present in the synonyms list too. See section "how can I insert a new synonym from Ranges".**

- Set **age**:
  - ↳ old = >60 years old;
  - ↳ adult = from 12 to 60 years old;
  - ↳ child = from 2 to 12 years old;
  - ↳ newborn = less of 2 years old.



**You can insert more detailed description, but remember that all of these descriptions MUST contain only ONE of these key words (old, adult, child or newborn). This rule is necessary for the correct execution of the query. If you don't insert one of these words, you can have problem with the execution of the validator and extractor, in which case you would have to check the database and delete the wrong range.**

- Set **gender**:
  - ↳ Female
  - ↳ Male
  - ↳ Female and male
- Set value of **Range**. Remember that you have to insert the minimum and maximum value and optionally the average.



**All the value in the database must be inserted in microM ( $\mu\text{M}$ ).**

- Optionally, you can insert into the **Note** area a note of up to 1000 characters. Notes can contain information on how you developed the experiment or all other information that can help the other users understand the origin of the data. It's a good rule to insert here all the information that is not directly

associated with the source. Source information must be inserted in the bibliography description.

- Click **“INSERT”**



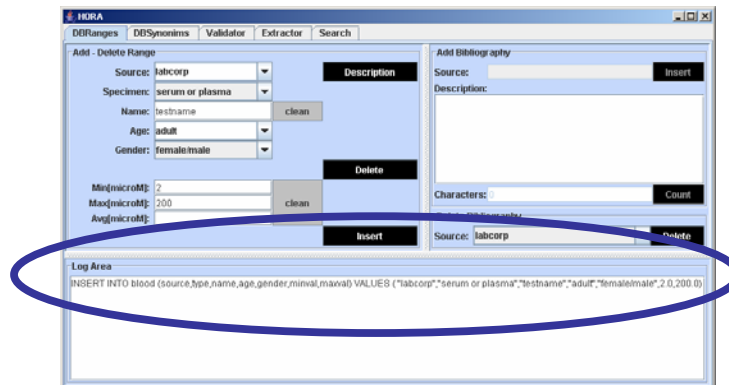
**The Ranges are stored in the database associated with source, name, specimen, age and gender, so you can't insert more than one range with all these attributes the same.**

### **Delete reference range**

In the panel “Add-Delete Range” you can delete a Range, just like when you insert a new one, using the following steps:

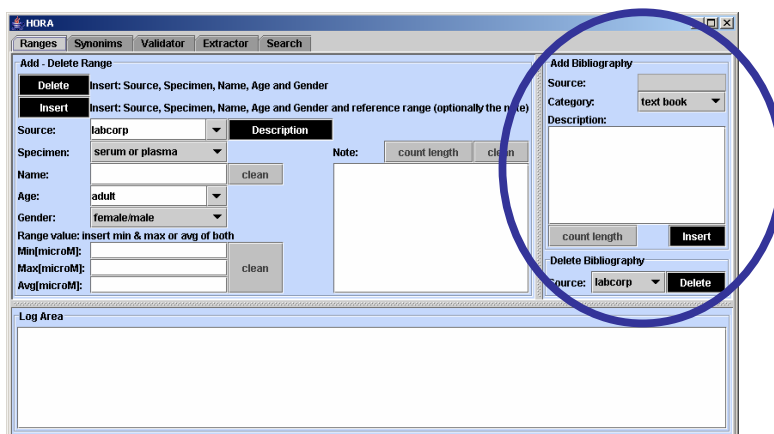
- Select the **source** from the list if the source is present in the bibliography database. If you want to insert a new source you can write the new name of the source, and following the instruction “Add new source” in the “Add Bibliography” panel.
- Set the **specimen**:
  - serum or plasma;
    - ↳ plasma;
    - ↳ serum;
    - ↳ blood - whole blood.
- Set **age**:
  - ↳ old = > 60 years old;
  - ↳ adult = from 12 to 60 years old;
  - ↳ child = from 2 to 12 years old;
  - ↳ newborn = less of 2 years old.
- Set **gender**:
  - ↳ Female;
  - ↳ Male;
  - ↳ Female and male.
- Click **“DELETE”**

## How do I know if the operation is successful?



If the operation is successful, HORA displays the information about the executed query in the Log Area so you can maintain a history of your commands, but remember that the Log Area is just a panel and is not designed to be permanently stored in any sort of file or backup that can be retrieved later.

## Bibliography

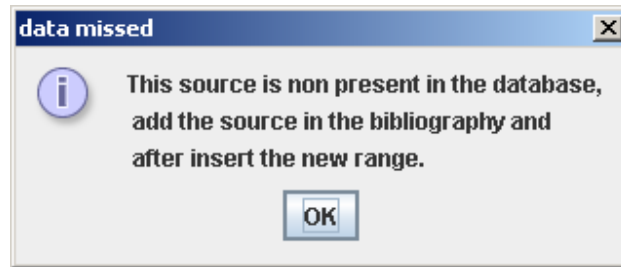


## How can I see the description of a source?

If you need to delete or insert a Range, but you wish to check the description to be sure that it is the correct source, you can click on the button **“DESCRIPTION”**. The description will be displayed in the “Log Area” panel.

## Insert new source in bibliography

When you try to insert a new normal value that references a source not present in the database, HORA stops you with this panel:



**You have to insert a new bibliography source before inserting a new value that references it.**

When you click on **OK**, the “Add Bibliography” panel will be activated and the new name will automatically be set in the **source** text area. At this point you have to choose the category of the source:

- Text book, i.e. literature references;
- Article, in this case it’s a good rule to insert only article published in scientific journals;
- Online database;
- Other contributions, like posters, personal data, articles from non-science journals.

Finally, you must insert a description of no more than 1000 characters (including spaces), and click **“INSERT”**. You can check if the length of the description is correct by clicking **“COUNT LENGTH”** and reading the result in the “Log Area”.

### **Delete source from bibliography**

To delete a source, select the name from the menu in the “Delete Bibliography” panel and click the **“DELETE”** button.

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## HORA - Graphic Interface - Synonyms Panel

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Through the Synonyms panel you can manage the information contained in the database that relates to the synonyms section.

It is divided in three panels:

- “Add Synonyms”, here you select which way to manage the data:
  - ↳ using the IUPAC code
  - ↳ using the PLBLD code
- “Set list of synonyms” where you can compile a list of synonyms and insert them into the database.
- “Log Area” where you can check the correct execution of the operation.

The screenshot shows the HORA software interface with the 'Synonyms' tab selected. The window title is 'HORA'. The interface is divided into three main sections:

- Add Synonyms - choose or from IUPAC code or CODE PLBLD:** This section contains two radio buttons. The first is selected: 'select insert synonyms from IUPAC (no use (""))'. The second is 'select insert synonyms from CODE (only if the code is present in the database): PLBLD'. There are text input fields for both options.
- Set list of synonyms:** This section has a 'Name' input field on the left and a 'List of synonyms:' input field on the right. Below the 'Name' field are 'Add' and 'Remove' buttons. Below the 'List of synonyms:' field are 'Insert' and 'Delete' buttons.
- Log Area:** A large empty rectangular area at the bottom of the panel.

### Insert list of synonym's names in the database

- In “Add Synonyms”, choose which type of way to manage the data and insert the requested information.
  - ↳ If you want to extend a group of synonyms already present in the database inserting a list of new name, you can use either the entry: the IUPAC or the PLBLD code.
  - ↳ If you want to create a new group of synonyms you must choose to insert using the IUPAC. In this way, you need to know the new IUPAC code that is not already present in the database. Once the new IUPAC and its synonyms are entered, HORA will create a new PLBLD code and link it to the new entries.

- Set the list of names.
- Click **“INSERT”**.



The description of IUPAC code can contain letters, numbers and symbols, but not quotation marks: “.

#### Delete list of synonym's names in the database

- In “Add Synonyms”, choose which type of way to manage the data and insert the information request.
- Set the list of name.
- Click **“DELETE”**.



After a deletion, if the PLBLD code no longer has an association with at least one name present in the synonyms table of the database, the connection between the PLBLD code and the respective IUPAC code is removed from the IUPAC table too.

#### How can I set the list of names?

To set the list you have to:

- insert the name in the area below the label “Name”;
- click **“ADD”** if you want to add it to the list or click **“REMOVE”** if you want to delete the name from the list.



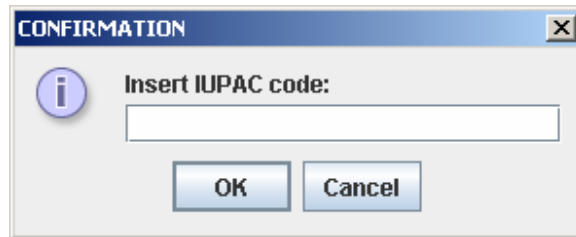
Name can contain letters, numbers and symbols, but not quotation marks: “.

#### How can I insert a new synonym from Ranges?

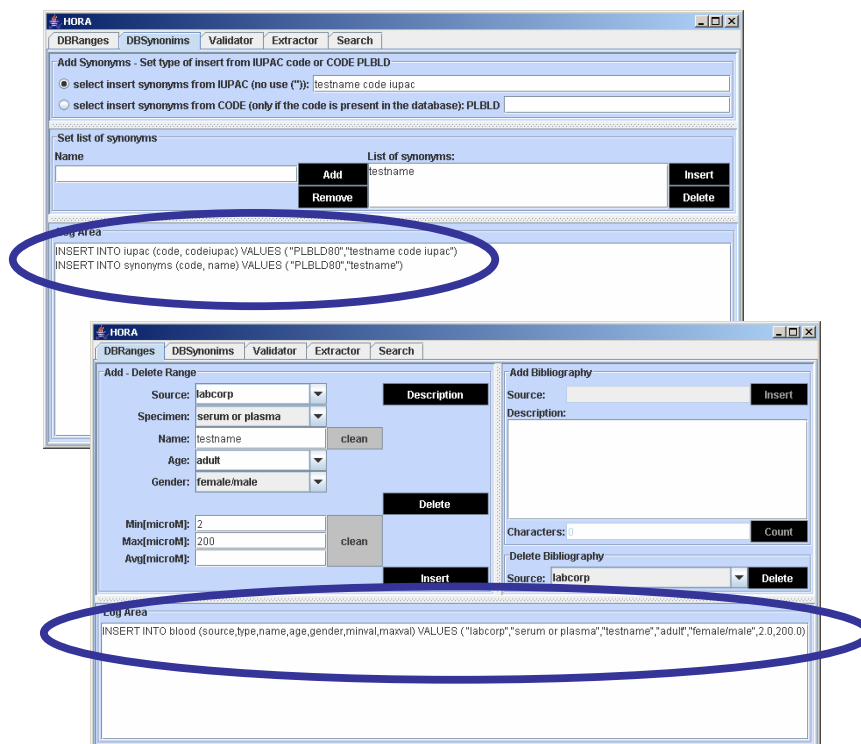
When you are working with the reference and you try to insert a new name (metabolite), which is not present in the synonyms section of the database, HORA stops the input and asks you if you want to insert this name in the synonyms list with this panel:



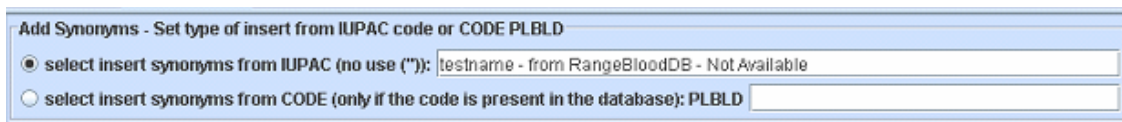
If you answer **NO**, HORA stops the operation and you can check to make sure that there was not a typing error. If you choose **YES**, you continue with the procedure, inserting the IUPAC code in the next panel:



You can abort the procedure like before by clicking **CANCEL**, or you can go on with it by clicking **OK**. HORA, in this case, sets all parameters in the Synonyms panel and automatically inserts the new reference range and the new synonym's group in the database, as showed in the "Log Area".



**If you don't know the IUPAC code, you can leave the IUPAC area blank and click **OK**, in this case HORA inserts a general string in the place of the IUPAC code: "[name metabolite] - - from RangeBloodDB - Not Available", as shown in the picture below.**



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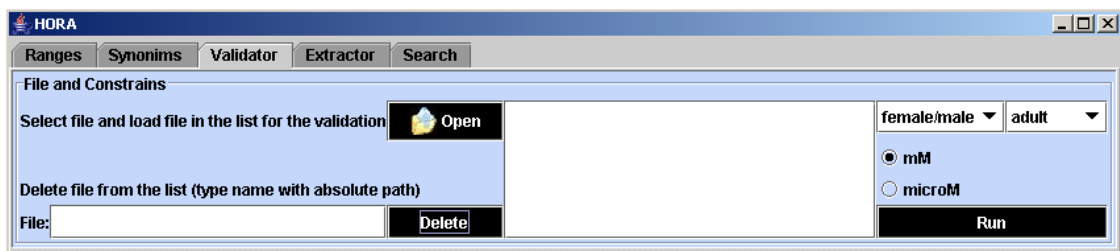
## HORA - Graphic Interface - Validator

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Through the Validator panel you can validate the concentration of metabolites listed in imported files.

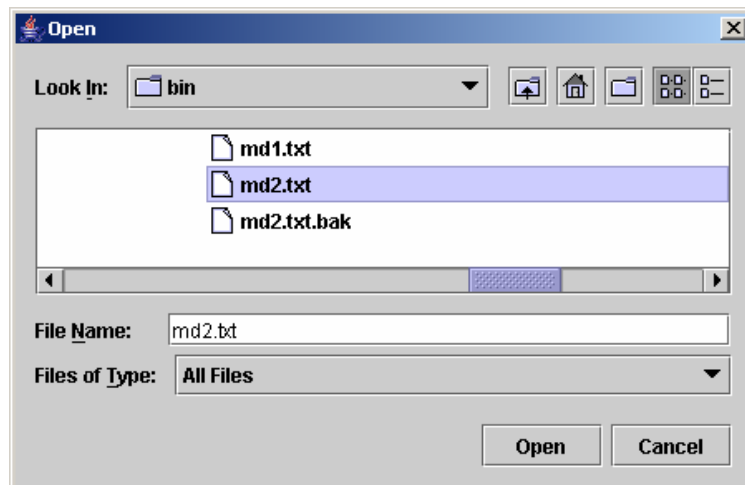
### Validation procedure

You can validate multiple files together as long as they use the same units of measurement.



In "File and Constrains" panel:

- Click **“OPEN”** to open the panel where you can navigate to find and load the file you wish to run. The path of the file will be written in the list to the right of this button. (You must repeat this for every file that you want analyzed concurrently)

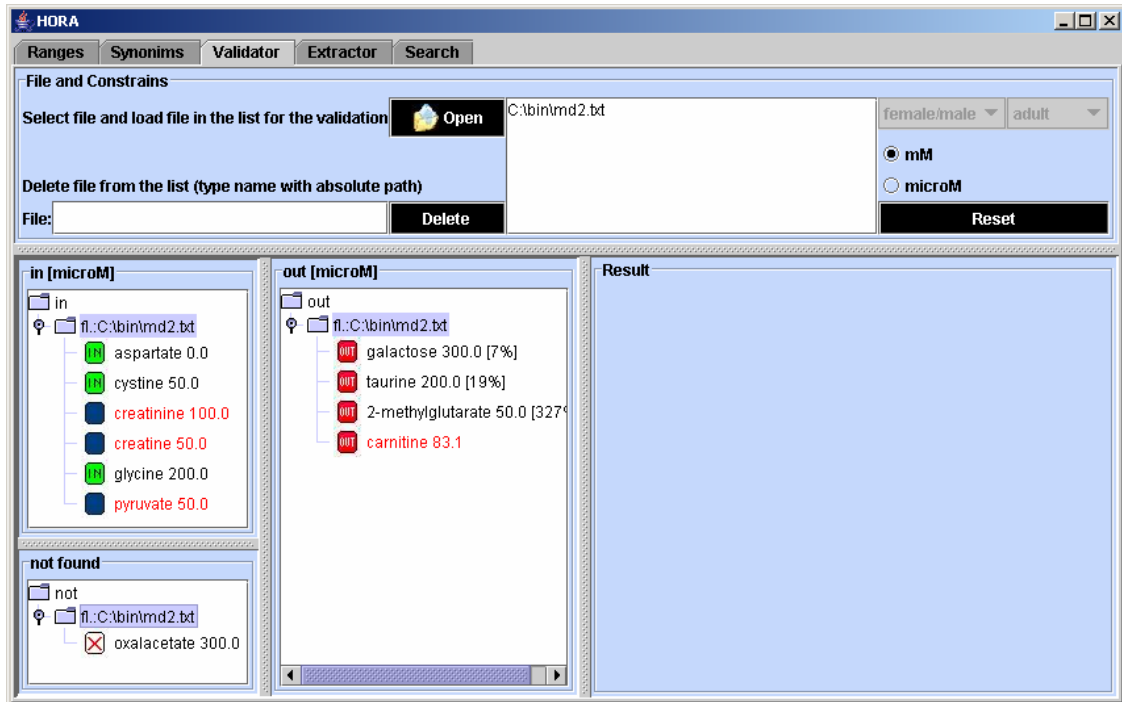


- If you want to delete a wrongly selected file, you have to type its path (as displayed in the list) in the space preceding the **“DELETE”** button. Clicking **“DELETE”** will remove it from the list.
- Set constrains of search:
  - ↳ gender;
  - ↳ age;
  - ↳ unit of measurement

- Click “**RUN**”.

At this point HORA starts the validation algorithm.

The results can be checked by navigating through the three trees constructed in the bottom left-hand area of the window, as shown in the picture below:



**All of the values displayed in the trees are shown in microM ( $\mu\text{M}$ ). The concentration is written after the name of the metabolite.**

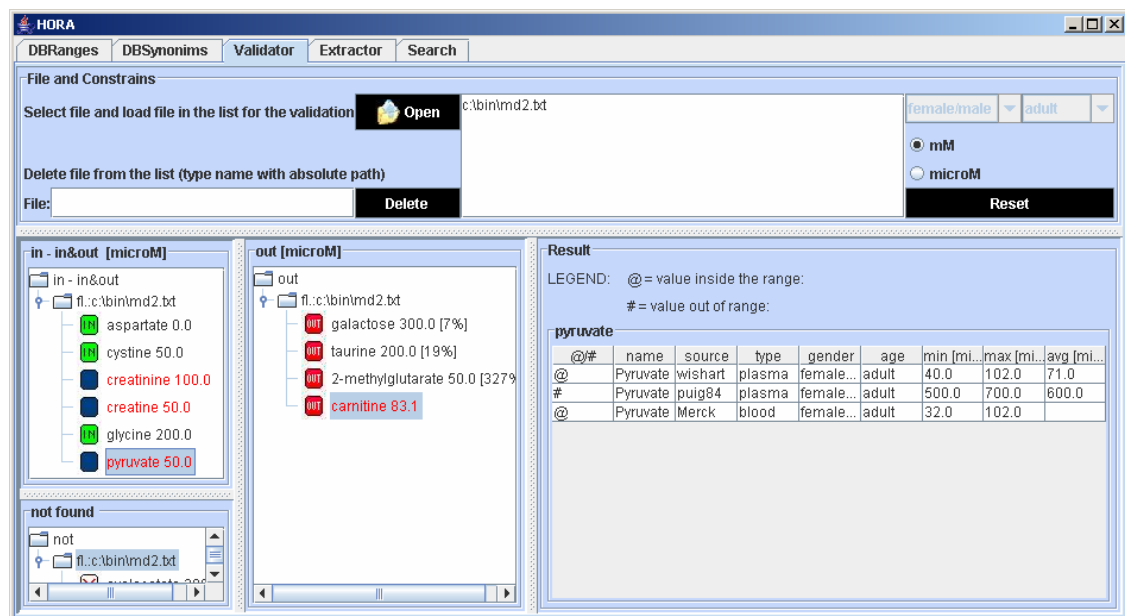
There are three different types of tree:

- The “in” tree contains:
  - ↪ These metabolites have concentrations that fall within **all** the ranges in the Database.
  - ↪ These metabolites have concentrations that fall within **some** of the ranges in the Database, but fall outside of at least one range. This is normal when a metabolite is specified from multiple sources. These cases are recognizable because the text will be displayed in red.
- The “out” tree contains:
  - ↪ These metabolites have the concentration out of the range contained in the Database.
  - ↪ The metabolites with this symbol that are **written in red** have a concentration outside of the range but only just barely (they have an overflow percentage of less than 0.5%). In this tree the overflow

percentage is listed after the name of the metabolite and the concentration.

- The “not found” tree contains,  the list of metabolites not found in the database. HORA is not able to validate these.

You can check the results and the range of the metabolites in the database by selecting a node in the tree and viewing the table that HORA creates for it in the “Result” area, as shown below.




The screenshot shows the HORA software interface. The main window has a menu bar with 'DBRanges', 'DBSynonyms', 'Validator', 'Extractor', and 'Search'. Below the menu is a 'File and Constrains' section with an 'Open' button and a file list containing 'c:\bin\md2.bt'. There are also 'Delete' and 'Reset' buttons. The interface is divided into three main panels:

- in - in&out [microM]:** A tree view showing metabolites with their concentrations and status icons (green for in-range, red for out-of-range). Metabolites include aspartate (0.0), cystine (50.0), creatinine (100.0), creatine (50.0), glycine (200.0), and pyruvate (50.0).
- out [microM]:** A tree view showing metabolites that are out of range. Metabolites include galactose (300.0 [7%]), taurine (200.0 [19%]), 2-methylglutarate (50.0 [327%]), and carnitine (83.1).
- not found:** A tree view showing metabolites not found in the database. It is currently empty.

The 'Result' panel shows a table for 'pyruvate' with the following data:

@/#	name	source	type	gender	age	min [mi...]	max [mi...]	avg [mi...]
@	Pyruvate	wishart	plasma	female...	adult	40.0	102.0	71.0
#	Pyruvate	puig84	plasma	female...	adult	500.0	700.0	600.0
@	Pyruvate	Merck	blood	female...	adult	32.0	102.0	






In the “Note” column, this symbol  means that there is a note written for that reference value. You can see it by clicking on the image.


### How can I say if the name in the Range area is the same of my list?


The validation algorithm checks all the names using the synonyms table, so if your list contains some metabolites expressed using synonyms found in that table, HORA recognizes that both names refer to the same metabolite.

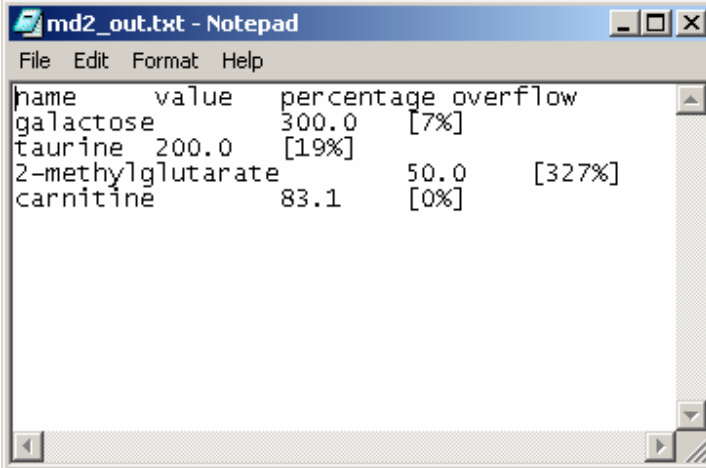
### Can I save the information of the validation?

For each file of the list of input, the three trees are saved in four different files in the same folder of the same file:

- one for  with extension \_in;
- one for  with extension \_in\_out;
- one for  with extension \_out;

- one for  with extension `_not`.

The structure of this file is the same of the file input, except for the  file where overflow percentages are written, and the values are separated with a tab (`\t`), as shown in the picture below.



```

name      value      percentage overflow
galactose 300.0      [7%]
taurine   200.0      [19%]
2-methylglutarate 50.0      [327%]
carnitine 83.1       [0%]


```

### How can I start a new Validation and clear the panel?

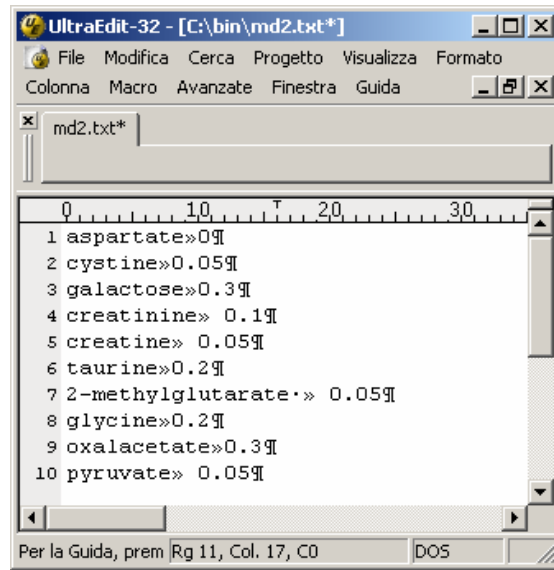
If you want to start a new validation you have to click **“RESET”** before restarting the validation procedure.

### How must the input file be structured?

The file must be:

- a `.txt` file
- structured in two columns
- divided with a tab (`\t`) , not a space.

The next picture shows one example:



This list can be generated using one of the various spectra analyzer or a common editor.

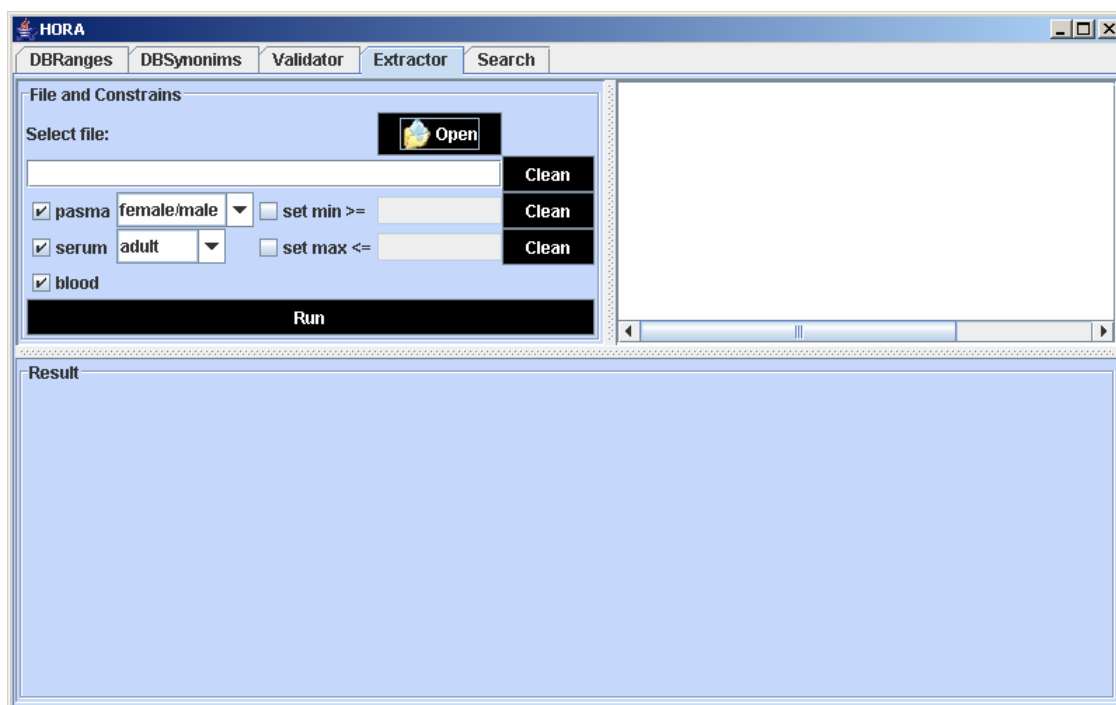
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## HORA - Graphic Interface - Extractor

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Through this panel you can extract from the database the metabolites that have a range that respects all constraints of a search, but are not present in the list that you found.

The goal is to give you a help to found other components that can be present but that you lost in your analysis.



### Extraction procedure

Follow the next step to extract the list of metabolites:

- open the file by clicking on “ OPEN”, the path or the file will be written in the dedicated area.
- Set the constrains:
  - ↳ specimen
  - ↳ gender
  - ↳ age

These are required.

- Optionally, you can set the range of search too. In this case you have to select to fix a minimum value or a maximum value or both and write a threshold (numerical value) in the followed area.
  - ↳ Fixing a minimum value means that the minimum value of a range must be more than the threshold.

- ↳ Fixing a maximum value means that the maximum value of a range must be less than the threshold.

- Click on button “**RUN**”.

### How can I see the list of metabolites extracted?

When the extractor algorithm is finished, HORA shows a table with all the results in the “Result” panel and writes the query executed in the predisposed area.


The screenshot shows the HORA software interface with the 'Extractor' tab selected. The 'File and Constrains' section includes a file selection field with 'c:\bin\md2.txt', a file type dropdown set to 'serum', and a gender dropdown set to 'adult'. There are checkboxes for 'pasma', 'serum', and 'blood', and a 'set max <= 100' field. A 'Run' button is visible. The 'Result' panel displays a table of extracted metabolites.

source	type	name	gender	age	min [microM]	max [microM]	avg [microM]
Merck	serum	Copper	female/male	adult	11.0	24.4	
Merck	serum	Cortisol	female/male	adult	0.11	0.607	
Merck	serum	Folic acid	female/male	adult	0.0	0.0043	
Merck	serum	Iron	female/male	adult	4.0	30.0	
Merck	serum	Mercury	female/male	adult	0.0	0.05	
Merck	serum	Progesterone	male	adult	0.0	0.0038	
Merck	serum	Vitamin A	female/male	adult	1.05	3.32	
Merck	serum	Vitamin B6	female/male	adult	0.03	0.144	
Merck	serum	Vitamin E	female/male	adult	12.0	46.0	
Henry01	serum	Acetoacetic acid	female/male	adult	20.0	100.0	
Henry01	serum	beta-Aminolevulinic acid	female/male	adult	0.8	2.3	
Henry01	serum	Arsenic	female/male	adult	0.0	0.4	
Henry01	serum	Carotene	female/male	adult	0.7	3.7	



Like the validator, the control on the list of the name is made using the analysis with the synonyms table.



If in the table, one row contains this symbol  in the “Note” column it means that there is a note written for this reference value. You can see it by clicking on the image.

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## HORA - Graphic Interface - Search

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Through this panel you can extract information using queries on the database for both Ranges and Synonyms. Obviously, the structure of the result differs depending upon the choice.


The screenshot shows the HORA software interface with the 'Search' tab selected. It features four search panels, each with a text input field and radio button options for search criteria. The panels are: Reference Range (with options for 'between min and max', 'as threshold [min>=T]', and 'as threshold [max<=T]'), Database Code "PLBLD" (with options for 'equal', 'Synonyms', 'like', and 'Reference Range'), IUPAC Code (with options for 'equal' and 'like'), and Name metabolite (with options for 'equal (with synonyms on "Range")', 'Synonyms', 'like (without synonyms on "Range")', and 'Reference Range'). Each panel has a 'Search' button. Below the search panels is a large empty area labeled 'Result'.

The query can be executed:

- Using the range of values;
- Using the PLBLD code
- Using the IUPAC description;
- Using the name of the metabolite.

In the case of the query on Synonyms, the IUPAC table and synonyms table are united so you can check for each name both the PLBLD code and IUPAC code.



If this symbol  appears in the "Note" column of the results table, it means that there is a note written for that reference value. You can see the note by clicking on the image.

### What is the difference between "like" and "equal"?

- Choosing "**EQUAL**" will return search results that match the query precisely. If you want the exact database data with respectively the PLBLD code, IUPAC description and name of metabolomite equal the string keyed in the area, in this case select "**EQUAL**";
- Choosing "**LIKE**" will return search results that include the string of characters used in the query at some point. For instance, if you query the PBLD database using the query "111" and select "**LIKE**", the results will include 111, 1111, 4111, 1110, 1113, etc.