Reference Comb:
Comb used to calibrate the scanner whose absorbance values have been established at Biogal using a master Cannon/Epson scanner.

Specimen Details:
Identification number or name for each sample corresponding to each individual tooth of the Comb.

TWAIN Compliant:
TWAIN (Technology Without An Interesting Name) is the acronym for a set of worldwide standards for software that processes data exchanged between graphics application programs and input devices such as scanners. A device driver based on these standards is called a TWAIN driver. TWAIN compliant applications can call up a TWAIN driver from within their own windows to scan images.

Information:

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COMBSCAN SOFTWARE

User Guide

Biogal Galed Labs.

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Contents

4. Administrator Utilities:
Administrators have the right to add new users and establish their entrance rights, set the scanner details and change the working parameters through the "Utilities" tab.
Changes in the contrast and brightness values at the "Scan" window can only be done by an administrator.

Adding a new user:
At the administrator utilities window click "User" and "Add" tabs. At the left upper corner enter the name, password and the access rights. Different levels of entry and usage can be set to users. The access options are as follows:

0 - No access right
1 - Read only
2 - Read and scan only
3 - Read, scan and export
4 - QC special (Biogal users only)
5 - Administrator

To delete a user simply highlight the user name in the list and click "Delete".

Changing working parameters - The options are:

Permit entry without a password.
Permit scanning without validation.
Permit scanning with some specimen ID missing.
Persistent Comb catalogue when clearing scan window.
Remember Comb catalogue number for the next CombScan session.

We strongly recommend the administrator account be used only to set working parameters and select scanner as described in "Before Validation" section. Afterwards the account 3 should be set to all users, so the parameters won't be accidentally changed.

X - Glossary

Absorbance:
A numerical value for the grey level of the spot itself detected by the image analysis module within the CombScan.

Calibration:
Every scanner has its own particular response to various colors and shades. It is important that all scanners, worldwide give comparable results to those obtained by a Cannon/Epson scanner used at Biogal.
This is achieved by calibration with the aid of the Reference Comb supplied with the CombScan.
When the Reference Comb is scanned, the results are compared with the data obtained from Biogal, computing a linear correlation between two sets of data and calculating the data transformation coefficients and producing results which are as near as possible to Biogal results.

Comb:
Based on a solid phase immunoassay principle, it is a plastic card shaped like a comb on which purified antigens are attached.

CombScale:
A calibrated color card included with the ImmunoComb® test kit. Refer to the kit user manual for instructions on how to use it.

Data:
Accumulated records of results of all scanned Combs. Can be easily browsed and reprinted.

Negative Control serum:
Also provided with the Immunocomb® kit, contains no antibodies of the specific disease being tested and should show no reaction.
For kits testing more than one antigen, if a negative control shows a reaction, not all the tests will be rendered invalid; only the antigens corresponding to the invalid Negative Control.

Positive Reference:
 Immunocomb® test kits that do not require Positive Control serum will include a Positive Reference spot on each tooth of the Comb. The Positive Reference is calibrated to the same absorbance limits of the Positive Control serum.
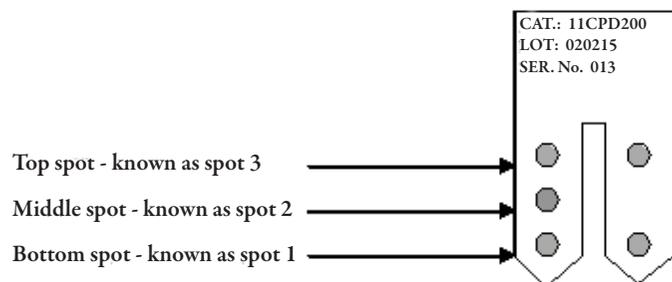
Internal Control:
In some Immunocomb® test kits the top spot is an Internal Control for the tooth. This monitors the correct performance of the test only. A very dark color reaction on the Internal Control spot, means that the reagents and color reaction are intact and an "OK" result is displayed. If a faint color reaction on the Internal Control spot occurs, a "NOT OK" result is displayed and the the results of that particular tooth will be invalid.

IX - Reference Material

1. Spot Indexing and Naming:
Within the literature accompanying the CombScan, you may encounter terms like top spot, lower spot, etc. A Comb may contain up to four spots per tooth. Bellow is an example for two teeth with three spots:

CAT.: 11CPD200
LOT: 020215
SER. No. 013

Top spot - known as spot 3

Middle spot - known as spot 2

Bottom spot - known as spot 1

2. Tooth Indexing (for a partial Comb):
When the index option is not used, the results of the scan performed will show the first tooth as No. 1, second tooth No. 2, etc (even though the actual numbers of the tooth in a partial Comb are for example 7 and 8).

3. Comb Details:
Consist of the catalogue number of the Comb, the lot and serial numbers. These numbers appear on three lines printed on the Comb.

The top line represents the **catalogue number** (**CAT**).
The middle line is the **lot number** (**LOT**).
The bottom line is the **serial number** of the Comb (**SER. No.**)

Is mandatory to enter the catalogue number but also strongly advisable to enter all the identifying numbers of the scanned Comb to maintain a proper record. In the event of queries to Biogal, the full details are required to identify the production lot.

I - Introduction

Reading the Immunocomb® results by eye has always been preferred as a simple and convenient method of interpretation of tests carried out in the field or veterinarian clinics.
For users who prefer an automated system, Biogal has developed the CombScan computerized system.

This new and improved version of CombScan software serves to provide objective quantitative or qualitative results of Biogal Immunocomb® tests. It does so by employing a TWAIN compliant scanner to scan the developed Comb. The data is then analyzed and interpreted to clinically meaningful results.

The image analysis is the heart of CombScan. It detects the faintest spots that are barely visible to the eyes. When a spot is detected, it is quantified and assigned a mean net grey level on a scale between 0 and 255. This value is termed "net absorbance". Substracting the background color from the color level of the spot itself, derives it. Therefore, every spot on the Comb receives a quantitative value along with a qualitative classification.

The qualitative classification is one of three values:

Non existence of spot	0
Existence of spot	Numerical value
Questionable spot result	Numerical value with purple background

II - Installing and Starting CombScan

Minimum Requirements:

Pentium PC with a USB connection.

Operating Systems:
Microsoft® Windows 98.
Microsoft® Windows 2000.
Microsoft® Windows XP.

Before installing the CombScan software make sure you have a scanner attached to the computer with its original software already installed.
A quick help presentation (power point) is available in our website www.biogal.co.il and can be also found on the Software Installation CD.

Insert CD into CD-ROM driver and wait until the installation box appears.
Select the target folder: "C:\Combscan2007" or "D:\Combscan2007".
Click install to start installation of CombScan software.

If the installation fails to appear:

On the desktop click the Start button at the lower left corner of the screen.
Click Run;
On the Run dialog box click Browse;
Select the CD-Rom driver,
Double click CombscanInstaller.exe;
Click OK.
Proceed with the installation as described above.
If this is the first time the CombScan software is being installed onto your system, click Close button to exit after finishing the installation.

If a previous version of CombScan software is found on your system, at the end of the installation a message will be presented: "**To complete the upgrade, you must now copy the file "CS2000Results. dat" from folder Combscan 2000 to folder Combscan 2007.**
This is a very important step to follow. By doing so, all the data stored on the previous version will be imported into the new one.

Creating a shortcut to the program:

Right-click on the "bgcs2007Proj.exe" file located in the newly created folder "C:\Combscan2007" or "D:\Combscan2007" (**Fig. 1**).
Select the option "Send to" and then choose Desktop (Create a shortcut). A shortcut will be placed on your desktop.
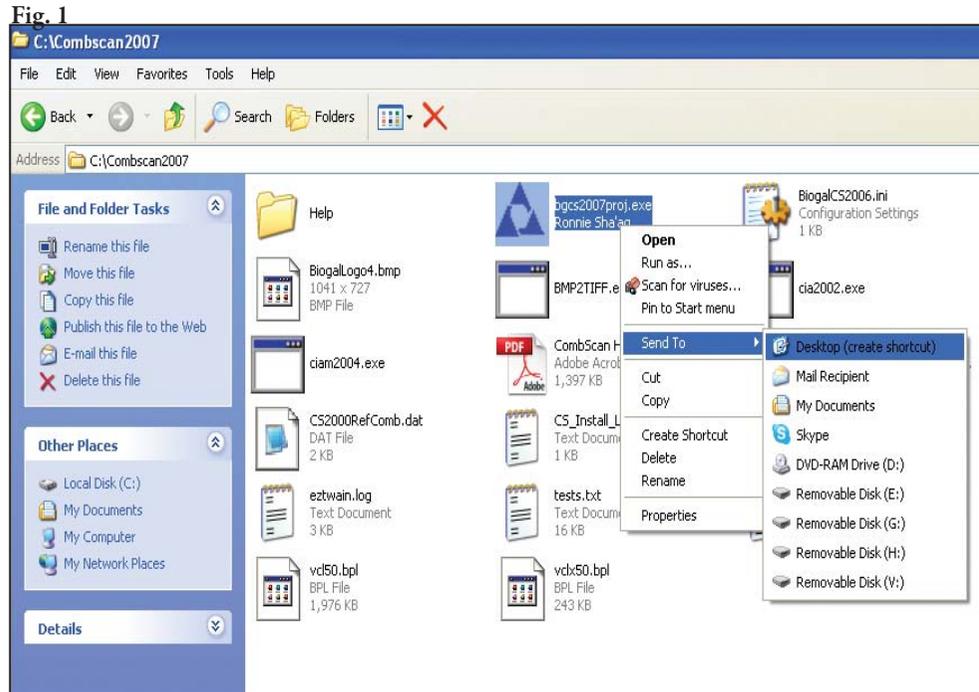
Fig. 1

III - Logging On To the System (LOGIN)

Click the bgcs2007Proj.exe file to start the program.
In the first screen click the "LOGIN" button.
Use the default Name and Password:
Name: **admin**
Password: **admin**

Before Validation (First Time Only)
Purpose: Select the scanner driver, find the right position of the Comb on the scanning plate and adjust contrast and brightness.

1- Selecting the right driver: (Fig. 2)

VII - Categories of Interpretation

The CombScan results are interpreted into 3 categories:

1. Net Absorbance:
The value of the grey spot on the tooth after its immediate background color has been subtracted.

2. Relative Absorbance:
The relative absorbance results are relevant to diagnosticians familiar to ELISA test reading methods, which are defined as:
(Absorbance of specimen X1000) / (Absorbance of Positive Control). Also known as S/P (sample/positive).
E.g., Sample 1: net absorbance = 26   positive control = 28
                         26/28=0.928
The relative absorbance for this sample would be 928.

3. Clinical Results:
Clinical results are the absorbance units translated into statistically correlated standard units of diagnostic measures such as MAT, IFA, CF, HI or VN, to assist the CombScan user interpreting the results in units they are familiar with.
The following example shows the Immunocomb® (IC) in relation to MAT results for Bovine *L. hardjo:*

IC	MAT
S0	0
S1	<1:50
S2	1:50
S3	1:100
S4	1:200
S5	1:400
S6	1:800
>S6	>1:800

Variability of Results:
Similar to all electro-optical measurement instruments, the CombScan exhibits some inherent variations in the Net Absorbance and Relative Absorbance results. By conducting repeated readings of each specimen in question, you can establish the standard deviation variation for each case.
A difference of 5% or less between two single readings, conducted on the same day and on the same scanner, is not statistically significant. Such readings should be considered identical.

VIII - Controls and References

Positive Control serum:
Provided with the Immunocomb® kit, defines the cutoff value and is always classified as "S3". For Clinical and/or Relative Absorbance results, the absorbance of the sample tested is compared to the absorbance of the Positive Control Serum.

Different numbers or names can be typed instead. Depending on the test type a *Positive Control and a *Negative Control are required. Enter a plus (+) sign for Positive Control and a minus (-) sign for Negative Control.

*See Chapter VIII page 9.

4. Place the Comb, results facing the glass, with the teeth pointing towards the center and away from the edge, in the right corner of the scanning glass*.
When scanning partial combs use at least 2 teeth. On the left side of the screen click "First Tooth Index" and "Last Tooth Index" arrows to select the first and last teeth numbers.

*Please Note: In the case of some scanners, the Comb will be scanned in a different corner. This can be checked on an individual basis. See section **2** on page **5**. In addition, brightness and contrast may vary and can be adjusted in the "Scan" window. See section **3** on page **5**.

5. Click the "Scan" tab. Click "OK" on the reminder message and wait a few seconds until the scanning process is finished.

Following a successful scanning procedure,   the results of the scan analysis will be automatically presented in the "Results" screen.
You can view Clinical results, Absorbance (Net absorbance) and Relative absorbance values by clicking the corresponding tabs at the bottom left side of the screen.
A green rectangle at the lower left corner of the screen indicates that the scanner has been successfully validated. A non validated scanner is indicated by a red rectangle.

6. Printing results:
In order to get a printout of the results click "Print" (bottom right side of the screen). The printout includes clinical results, absorbance and relative absorbance values.

7. Saving Results:
Results can be saved by clicking the "Export" tab. In the next window choose the parameters to export by checking the appropriated box and the number of records to export. The records are saved in both **TXT** and **CSV** formats and can be found on the CombScan directory. After exporting the records click "Cancel" to return to the previous screen.

8. Combscore:
Is a tabulation, which produces a histogram (bar graph), representing the antibody titer of the flock. Click the "Combscore" tab, at the next window stipulate the number of combs to be included in the Combscore group, enter the group/flock name/ID and click OK. The Combscore can be printed and/or exported into a **TXT** file format.

VI - Interpretation of Results

Interpretation of results varies according to the type of test, as explained in details in the "Reading and Interpretation of Results" section of the Instruction Manual enclosed with each Immunocomb® antibody test kit.

Absorbance:
The CombScan measures the color intensity of the Immunocomb® test results in absorbance units. The absorbency values measure the amount of light absorbed by a colored surface. The CombScan detects and reads the absorbency of each spot on the developed Comb.
The color of each spot is calculated on a scale of 0-255, where white shown as 0 is the minimum absorbency, through varying shades of grey to black shown as 255 which is the maximum absorbency.

*The CombScan software works only with fully **TWAIN** compliant devices. Make sure the scanner being used is **TWAIN** compliant.
At Main Menu screen, click the "Utilities" tab and at the next screen click on the "Scanner" tab. In the "Select Source" window select the scanner's driver. Highlight the **TWAIN** driver. Click "Select". Click "Close" to exit to the main window.

Fig. 2
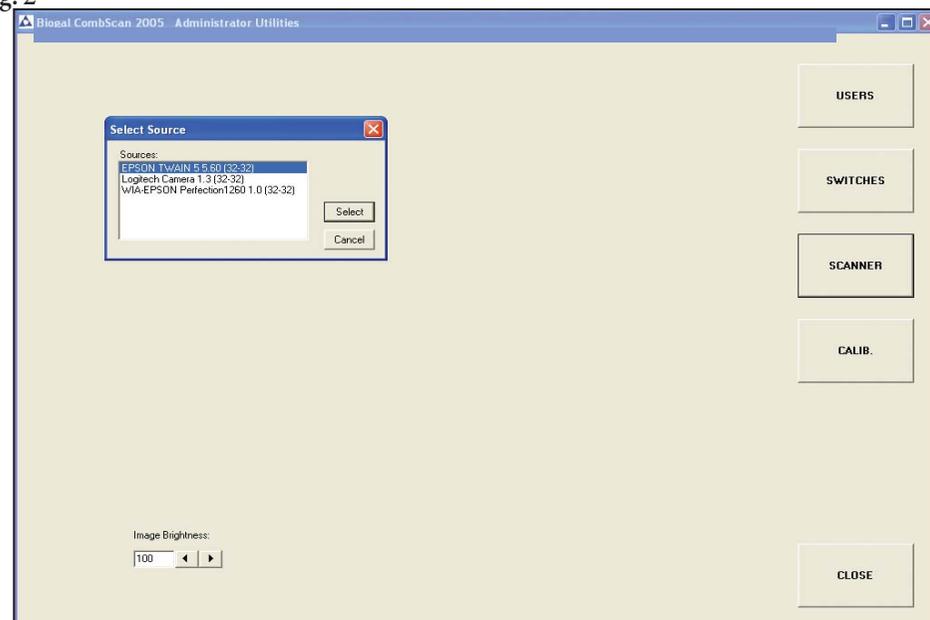
2- Finding the right position of the comb on the plate:

At the main menu, click on the "Scan" tab. At the next window enter the details of the **Reference Comb** that came with the software (i.e. catalog number, lot number, serial number) (**Fig.3, page 6**). Place the Comb printed side down in one of the corners of the scanner. Make sure the teeth are pointing toward the center of the scanning plate. Place a black sheet on top of the Comb once it has been placed on the glass. (The background must be black) Please, always use a black background to cover the Comb. Leaving the scanner cover open, won't achieve the same result. Click the "Scan" button to activate the scanner and the image analysis module. At the end of scanning a results window will be presented. Click the "Inspect Image" button.

● If only part of the Comb can be seen, click "Close" to return to previous window and change the position of the Comb on the plate. Retry scanning.
● If you cannot see any part of the Comb, move it to a different corner of the scanning plate and retry scanning.
● Follow the above steps until finding the position. Once this is done, the entire Comb image will be presented. (**Fig. 4, page 6**)
● If an "Image File Blank" or "Problem with the shape of tooth —- (random number) in the scanned comb" messages are presented; see the next step "Adjusting contrast and brightness".

3- Adjusting contrast and brightness (**Fig.3, page 6**)
The contrast and brightness default parameters are:
Brightness: **100**
Contrast: **0**

At the scan window in the scanning parameters box type the new values to increase or decrease contrast and brightness and scan the comb again. Inspect the Comb image for changes in contrast and/or brightness.

• If after several trials there are still no changes in the Comb image, check if program is using the right scanner driver, as described in the "Selecting the Scanner's driver" step.
• If you are not sure which driver should be used or if the scanner is TWAIN compliant device, check the manufacture's papers
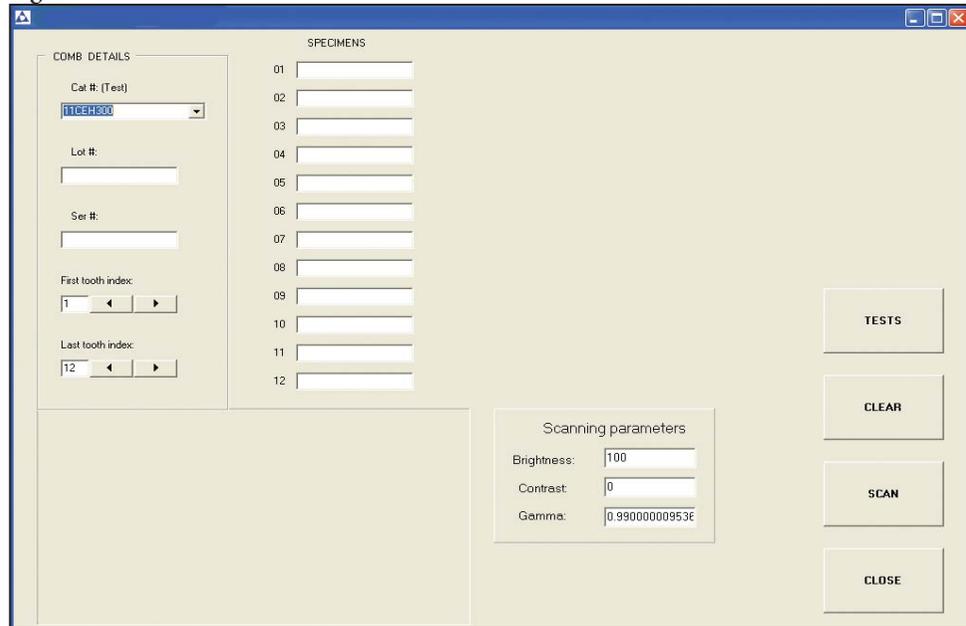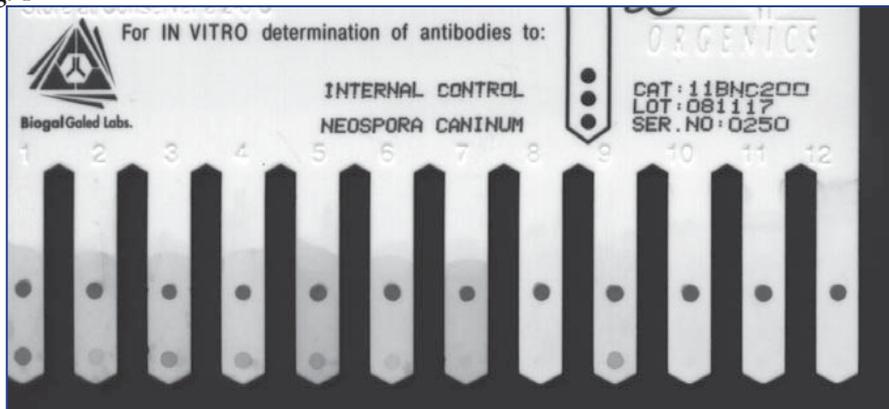
Fig. 3

Fig. 4

IV - Validating the System

To confirm the accuracy and stability of the CombScan system, it is recommended that **Validation** be performed daily before scanning combs.
An invalidated CombScan system may give inaccurate results. In such cases, subsequent scan and results will show an indication that the CombScan has not been validated.
Scanning a Reference Comb, provided with the CombScan package, performs validation and calibration. This Reference Comb has been scanned at Biogal-Galed Labs using a master reference Canon/Epson scanner.
At the main menu screen click the "Validate" tab:
1- Enter Reference Comb details: Catalogue number, lot and serial numbers.
2- Place the Comb in the correct position in the scanning plate placing a black sheet on top of it.
3- Click on the "Scan" tab. Click OK to the reminder message.
4- Validation results will be presented. Click OK for all the pop up messages. A message of "Success" or "Failed" will be presented according to validation results. (Fig. 5)
5- If validation is successful the scanner is ready for use. Proceed to scan Combs.
6- If validation process failed check the position of the Comb and that the correct Reference Comb is being used, change the contrast and brightness as seen on chapter III-3.
7- If validation fails after several trials, contact your distributor or Biogal by e-mail: info@biogal.co.il or by phone: 972-4-9898605.

Fig. 5
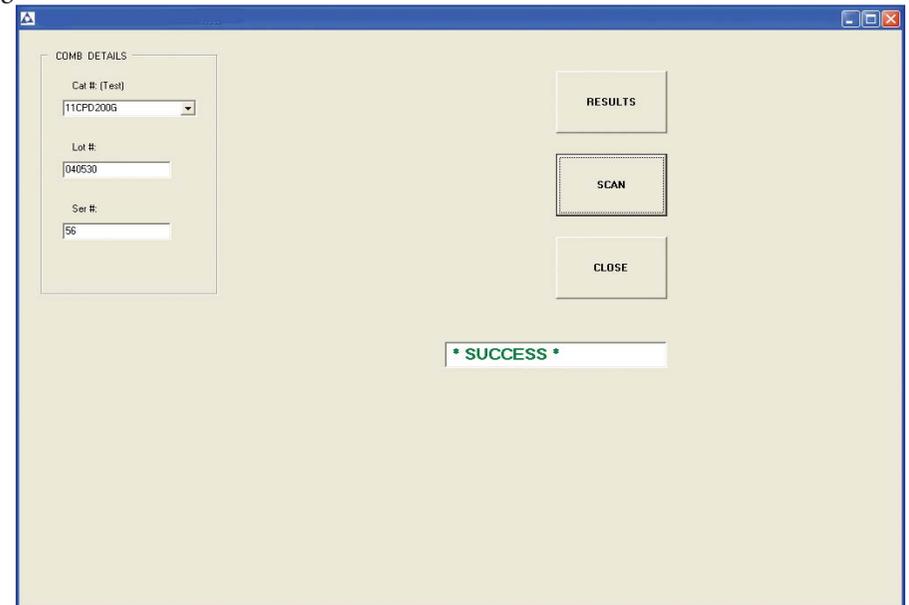
V - Using the Program to Scan Combs

Once the scanner has been validated, it is ready for scanning and analyzing Combs.

1. From the main menu select "Scan".
2. In the next window fill in the Comb details.
3. Enter the Specimen details in the corresponding specimens boxes hitting "Enter" after each specimen entry. The specimen numbers 1-12 are given as default.