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Case study: Interpretation of Hepatitis B Serological Test Results for Vaccine susceptibility (Hypothetical Adaptation for the Microarray Assay)

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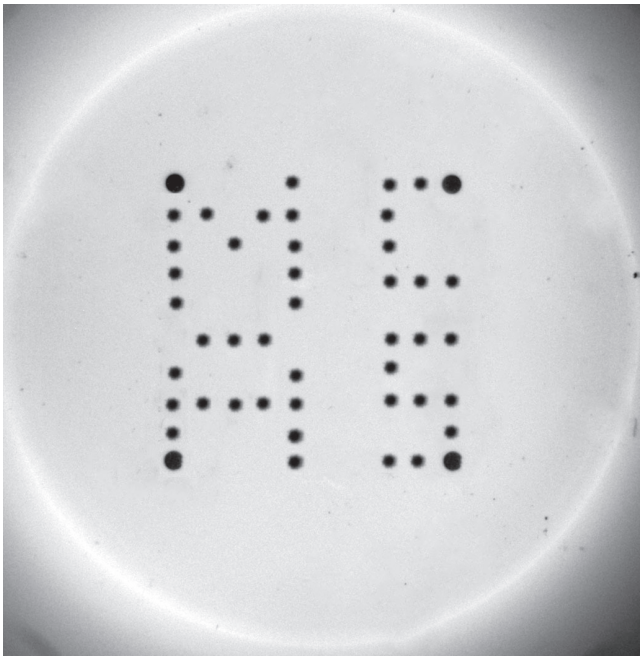
Abstract

The case study here would be the CDC Adaptation from: A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. Part I: Immunization of Infants, Children, and Adolescents. MMWR 2005;54(No. RR-16).

The purpose of this Case study would be to show the process of creation and use of this assay with Colorwatcher, micro array imager and QuantoPic software.

This study does not cover nor the creation of the assay techniques on the microarray spotter, nor principles of the ELISA, nor sample preparation, nor details on the nature of any markers.



Microarray and Assay design

During imunnogenesis of HBV following clinically significant markers can be found: Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (anti-HBs), Total hepatitis B core antibody (anti-HBc), IgM antibody to hepatitis B core antigen (IgM anti-HBc) (www.cdc.gov/hepatitis).

For better robustness of validation we design the array so it would be visually readable. So we have created a 10x10 array, where the spots are placed in letter shapes. HBsAg = A , anti-HBs = S , anti-HBc = C , IgM anti-HBc = M. Each visually readable letter results as a positive ELISA reaction. Positive controls are located on the corners of the array.

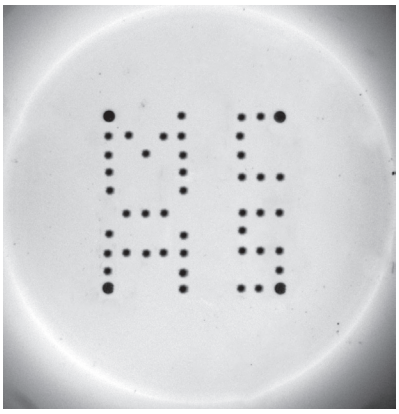
For the assay we will also use a positive and negative wells.

	1	2	3	4	5	6	7	8	9	10
1	PC P1				IgM			HBc	HBc	PC P1
	54180	21312	21150	20862	49448	20535	20895	49480	49403	52190
2	IgM	IgM		IgM	IgM			HBc		
	48811	49525	21050	49366	49454	20700	20794	49367	20571	20841
3	IgM		IgM		IgM			HBc		
	48952	21184	49387	20829	49394	21132	20409	49019	20331	20861
4	IgM				IgM			HBc	HBc	HBc
	47748	21195	20987	21833	49381	20331	20418	49356	49460	49426
5	IgM				IgM					
	49474	21064	20838	21020	50984	20296	20153	20096	20460	20865
6		HBs Ag	HBs Ag	HBs Ag				HBs	HBs	HBs
	21520	49045	49445	49415	20226	20314	20242	49427	49427	49148
7	HBs Ag				HBs Ag			HBs		
	49501	20777	20449	20126	50977	20082	20070	50922	19979	20800
8	HBs Ag	HBs Ag	HBs Ag	HBs Ag	HBs Ag			HBs	HBs	HBs
	48866	48908	48742	49433	49427	20124	19835	49041	49005	49139
9	HBs Ag				HBs Ag					HBs
	49037	20523	20397	19830	49403	19730	19591	19624	20096	49098
10	PC P1				HBs Ag			HBs	HBs	PC P1
	51705	20118	19807	19731	49409	19992	19821	49036	50977	51855

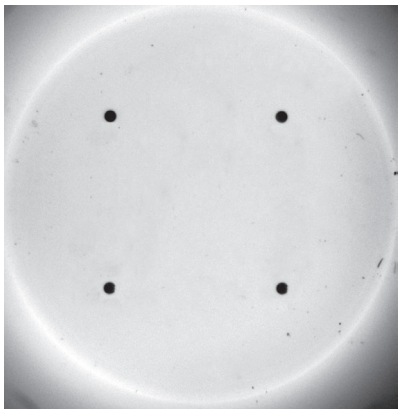
Imaging

We are using a Colorwatcher, microarray imager for obtaining the images of an 8 well strip.

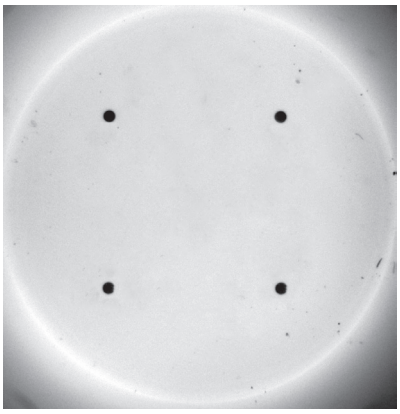
a1, positive control well



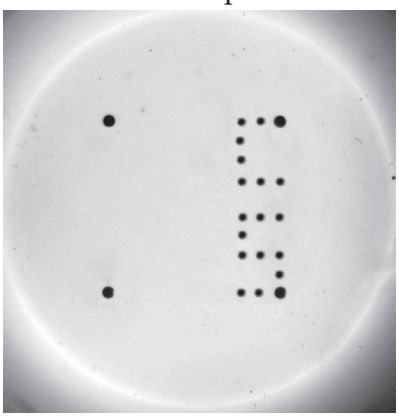
b1, negative control well



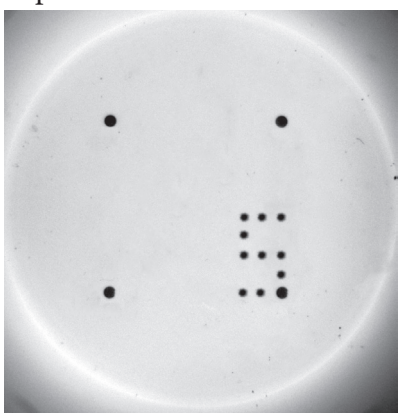
c1, susceptible patient



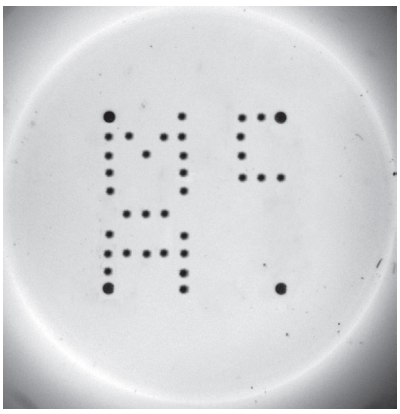
d1, Immune due to natural infection patient



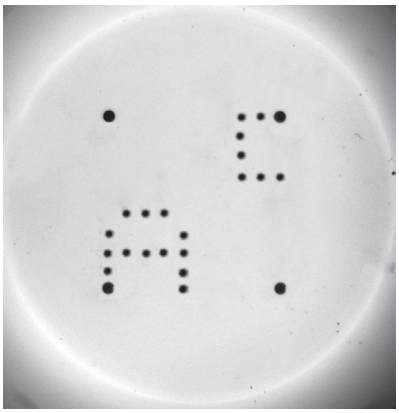
e1, Immune due to hepatitis B vaccination



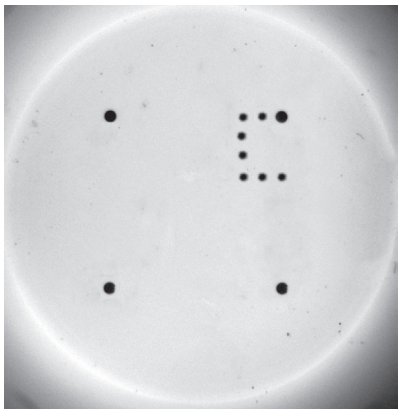
e1, Acutely infected



g1, Chronically infected



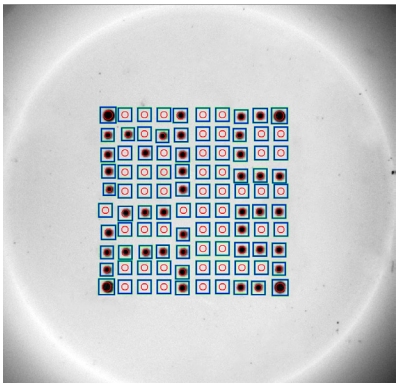
h1, Interpretation unclear: Resolved or other



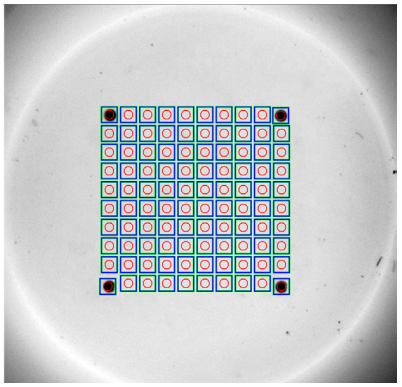
Autofind

Manual array placement is a tedious task which is the microarray technology's repel factor, thus an easy, fast, smart and robust method is required. The QuantoPic software features the Autofind feature, which, by using highly complexed AI methods, which does the following: locates all the spots of interest; finds out the corner points of the array; places the microarray map. Below are the results of the automatic spot finding and map placement.

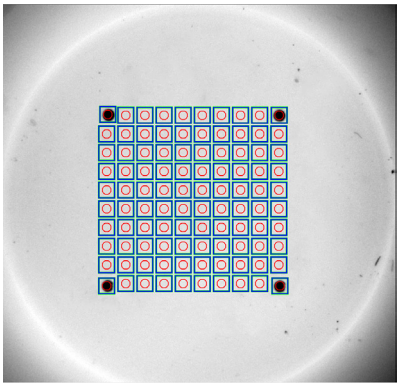
a1, positive control well



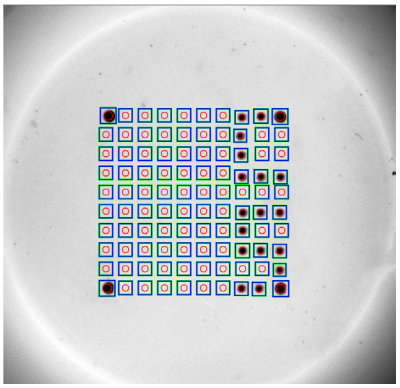
b1, negative control well



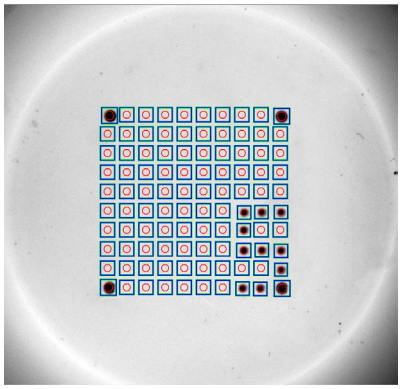
c1, susceptible patient



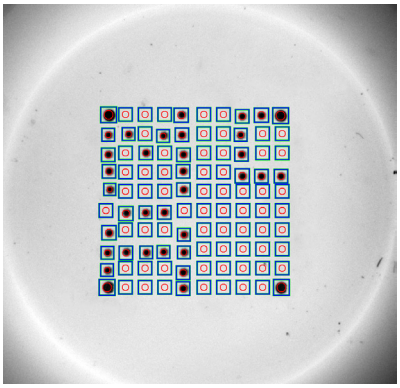
d1, Immune due to natural infection patient



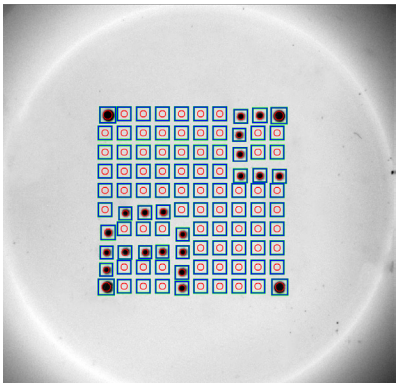
e1, Immune due to hepatitis B vaccination



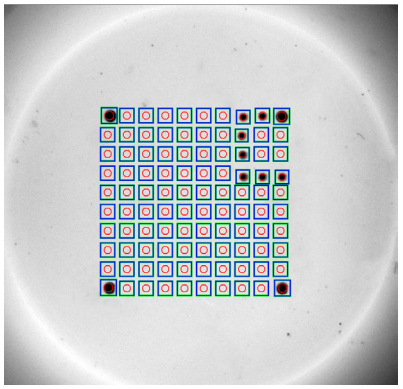
e1, Acutely infected



g1, Chronically infected



h1, Interpretation unclear: Resolved or other



Assay Creation

For creation of assay we need to identify the list of samples, as well as the number of multiplex groups. For this assay we will use 3 types of wells: Positive Control, Negative Control and Patient's wells. In each of the wells we would want to analyse all types of samples separately from each other. Below are 3 tables of samples, with its name and variable name in the software.

Table 1a, Positive control well

Sample	Variable
PC for IgM	[P1_0]
PC for Antigen	[P2_0]
PC for IgG for HBc	[P3_0]
PC for IgG for HBs	[P4_0]
PC in Positive Well	[P5_0]

Table 2a, Negative control well

Sample	Variable
NC for IgM	[N1_0]
NC for Antigen	[N2_0]
NC for IgG for HBc	[N3_0]
NC for IgG for HBs	[N4_0]
PC in Negative Well	[P6_0]

Table 3a, Patient's well

Sample	Variable
Patients IgM	[1T_0]
Patients Antigen	[2T_1]
Patients IgG for HBc	[3T_2]
Patients IgG for HBs	[4T_3]
PC in Patients Well	[P7_0]

Table 1b, Positive control well in software

	1	2	3	4	5	6	7	8	9	10
1	PC Pw [P5_0]				PC IgM [P4_0]			PC HBc [P2_0]	PC HBc [P2_0]	PC Pw [P5_0]
2	PC IgM [P4_0]	PC IgM [P4_0]		PC IgM [P4_0]	PC IgM [P4_0]			PC HBc [P2_0]		
3	PC IgM [P4_0]		PC IgM [P4_0]		PC IgM [P4_0]			PC HBc [P2_0]		
4	PC IgM [P4_0]				PC IgM [P4_0]			PC HBc [P2_0]	PC HBc [P2_0]	PC HBc [P2_0]
5	PC IgM [P4_0]				PC IgM [P4_0]					
6		PC Ag [P1_0]	PC Ag [P1_0]	PC Ag [P1_0]				PC HBs [P3_0]	PC HBs [P3_0]	PC HBs [P3_0]
7	PC Ag [P1_0]				PC Ag [P1_0]			PC HBs [P3_0]		
8	PC Ag [P1_0]	PC Ag [P1_0]	PC Ag [P1_0]	PC Ag [P1_0]	PC Ag [P1_0]			PC HBs [P3_0]	PC HBs [P3_0]	PC HBs [P3_0]
9	PC Ag [P1_0]				PC Ag [P1_0]					PC HBs [P3_0]
10	PC Pw [P5_0]				PC Ag [P1_0]			PC HBs [P3_0]	PC HBs [P3_0]	PC Pw [P5_0]

Table 2b, Negative control well in software

	1	2	3	4	5	6	7	8	9	10
1	PC Nw [P6_0]				NC IgM [N1_0]			NC HBc [N3_0]	NC HBc [N3_0]	PC Nw [P6_0]
2	NC IgM [N1_0]	NC IgM [N1_0]		NC IgM [N1_0]	NC IgM [N1_0]			NC HBc [N3_0]		
3	NC IgM [N1_0]		NC IgM [N1_0]		NC IgM [N1_0]			NC HBc [N3_0]		
4	NC IgM [N1_0]				NC IgM [N1_0]			NC HBc [N3_0]	NC HBc [N3_0]	NC HBc [N3_0]
5	NC IgM [N1_0]				NC IgM [N1_0]					
6		NC Ag [N2_0]	NC Ag [N2_0]	NC Ag [N2_0]				NC HBs [N4_0]	NC HBs [N4_0]	NC HBs [N4_0]
7	NC Ag [N2_0]				NC Ag [N2_0]			NC HBs [N4_0]		
8	NC Ag [N2_0]	NC Ag [N2_0]	NC Ag [N2_0]	NC Ag [N2_0]	NC Ag [N2_0]			NC HBs [N4_0]	NC HBs [N4_0]	NC HBs [N4_0]
9	NC Ag [N2_0]				NC Ag [N2_0]					NC HBs [N4_0]
10	PC Nw [P6_0]				NC Ag [N2_0]			NC HBs [N4_0]	NC HBs [N4_0]	PC Nw [P6_0]

Table 3b, Patients well in the software

	1	2	3	4	5	6	7	8	9	10
1	PC Pat [P7_0]				IgM [1T_0]			HBc [3T_2]	HBc [3T_2]	PC Pat [P7_0]
2	IgM [1T_0]	IgM [1T_0]		IgM [1T_0]	IgM [1T_0]			HBc [3T_2]		
3	IgM [1T_0]		IgM [1T_0]		IgM [1T_0]			HBc [3T_2]		
4	IgM [1T_0]				IgM [1T_0]			HBc [3T_2]	HBc [3T_2]	HBc [3T_2]
5	IgM [1T_0]				IgM [1T_0]					
6		Ag [2T_1]	Ag [2T_1]	Ag [2T_1]				HBs [4T_3]	HBs [4T_3]	HBs [4T_3]
7	Ag [2T_1]				Ag [2T_1]			HBs [4T_3]		
8	Ag [2T_1]	Ag [2T_1]	Ag [2T_1]	Ag [2T_1]	Ag [2T_1]			HBs [4T_3]	HBs [4T_3]	HBs [4T_3]
9	Ag [2T_1]				Ag [2T_1]					HBs [4T_3]
10	PC Pat [P7_0]				Ag [2T_1]			HBs [4T_3]	HBs [4T_3]	PC Pat [P7_0]

Results Interpretation

The next step would be to assign the positivity criteria and assign the results interpretation logic.
There are a few methods what units to use for the interpretation. The raw value obtained from a spot is number from 0 to 65 535. As well's surface transparency/reflection properties can vary for different manufacturers, the more robust method for getting a value would be to use a signal to noise ratio. Imperically the most robust formula we think is: Spot signal divided by it's background plus 3 Standard deviations of the background. For easier visual perception this value should be translated to percent. If the results is greater than 100%, then the sample is considered positive. In the software we have also choose to see the calculated number used in the interpretation.

Table 4a, Variables table

Sample	Variable	Formula
PC for IgM	[W1]	[P1_0]/([P1_B]+3*[P1_BS])*100
PC for Antigen	[W2]	[P2_0]/([P2_B]+3*[P2_BS])*100
PC for IgG for HBc	[W3]	[P3_0]/([P3_B]+3*[P3_BS])*100
PC for IgG for HBs	[W4]	[P4_0]/([P4_B]+3*[P4_BS])*100
PC in Positive Well	[W5]	[P5_0]/([P5_B]+3*[P5_BS])*100
NC for IgM	[W8]	[N1_0]/([N1_B]+3*[N1_BS])*100
NC for Antigen	[W9]	[N2_0]/([N2_B]+3*[N2_BS])*100
NC for IgG for HBc	[W10]	[N3_0]/([N3_B]+3*[N3_BS])*100
NC for IgG for HBs	[W11]	[N4_0]/([N4_B]+3*[N4_BS])*100<100
PC in Negative Well	[W6]	[P6_0]/([P6_B]+3*[P6_BS])*100>100
Patients IgM	[C]	[1T_0]/([1T_0_B]+3*[1T_0_BS])*100>100
Patients Antigen	[C_1]	[2T_1]/([2T_1_B]+3*[2T_1_BS])*100>100
Patients IgG for HBc	[C_2]	[3T_2]/([3T_2_B]+3*[3T_2_BS])*100>100
Patients IgG for HBs	[C_3]	[4T_3]/([4T_3_B]+3*[4T_3_BS])*100>100
PC in Patients Well	[W7]	[P7_0]/([P7_B]+3*[P7_BS])*100>100

Table 4b, Results interpretation for quality control and patients' samples

Sample	Conditional	True	False
PC for IgM	[W1]>100	Ok	Error
PC for Antigen	[W2]>100	Ok	Error
PC for IgG for HBc	[W3]>100	Ok	Error
PC for IgG for HBs	[W4]>100	Ok	Error
PC in Positive Well	[W5]>100	Ok	Error
NC for IgM	[W8]<=100	Ok	Error
NC for Antigen	[W9]<=100	Ok	Error
NC for IgG for HBc	[W10]<=100	Ok	Error
NC for IgG for HBs	[W11]<=100	Ok	Error
PC in Negative Well	[W6]>100	Ok	Error
Patients IgM	[C]>100	Positive	Negative
Patients Antigen	[C_1]>100	Positive	Negative
Patients IgG for HBc	[C_2]>100	Positive	Negative
Patients IgG for HBs	[C_3]>100	Positive	Negative
PC in Patients Well	[W7]>100	Ok	Error

$$\frac{Spot_{Signal}}{Spot_{Background} + 3 \times SD_{Background}} \times 100$$

Quality Control

The next step would be to assign the quality control. The usual mix we observe is, if either sample in positive or negative in control wells fail, indicate such an error. On the other-hand if all is ok, then indicate so.

Sample	Conditional	True
PC well	[W1] <= 100 || [W2] <= 100 || [W3] <= 100 || [W4] <= 100 || [W5] <=100	PC Error
PC well	[W1] >100 && [W2] >100 && [W3] >100 && [W4] >100 && [W5] > 100	PC OK
NC well	[W8] >100 || [W9] >100 || [W10] >100 || [W11] >100	NC Error
NC well	[W8] <= 100 && [W9] <= 100 && [W10] <= 100 && [W11] <= 100	NC OK

Diagnosis Interpretation

The next step would be to assign the diagnosis interpretation based on the Diagnosis interpretation table from the source.

Table 5b, Diagnosis interpretation table in QuantoPic

Conditional	Diagnosis
[W7]<=100	PC Error
[C_1] <= 100 && [C_2] <= 100 && [C_3] <= 100	Susceptible
[C_1] <= 100 && [C_2] > 100 && [C_3] > 100	Immune due to natural infection
[C_1] <= 100 && [C_2] <= 100 && [C_3] > 100	Immune due to hepatitis B vaccination
[C] > 100 && [C_1] > 100 && [C_2] > 100 && [C_3] <= 100	Acutely infected
[C] <= 100 && [C_1] > 100 && [C_2] > 100 && [C_3] <= 100	Chronically infected
[C_1] <= 100 && [C_2] > 100 && [C_3] <= 100	Interpretation unclear: Resolved or other

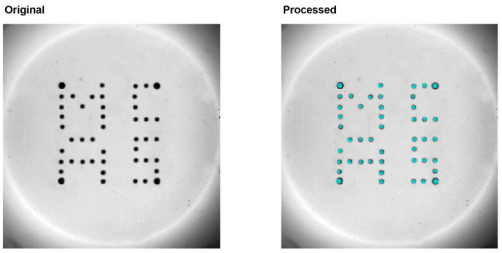
Table 5a, Diagnosis interpretation table

HBsAg **anti-HBc** **anti-HBs**	negative negative negative	Susceptible
HBsAg **anti-HBc** **anti-HBs**	negative positive positive	Immune due to natural infection
HBsAg **anti-HBc** **anti-HBs**	negative negative positive	Immune due to hepatitis B vaccination
HBsAg **anti-HBc** IgM anti-HBc **anti-HBs**	positive positive positive negative	Acutely infected
HBsAg **anti-HBc** IgM anti-HBc **anti-HBs**	positive positive negative negative	Chronically infected
HBsAg **anti-HBc** **anti-HBs**	negative positive negative	Interpretation unclear: Resolved or other

Results

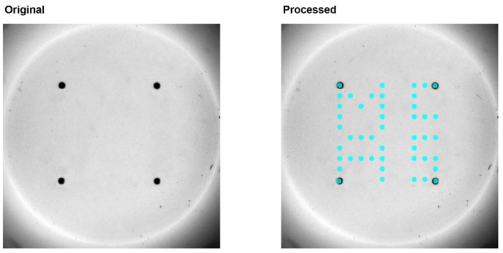
In this section we show the pages from the PDF report file.

a1, positive control well



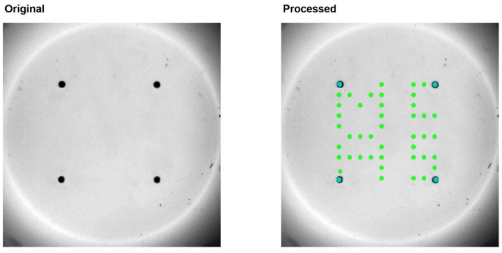
Test Diagnosis	Result1
HBV 2021-04	PC Ok

b1, negative control well



Test Diagnosis	Result1
HBV 2021-04	NC Ok

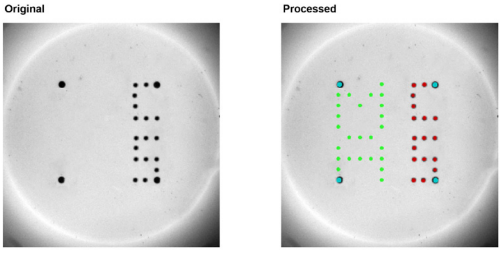
c1, susceptible patient



Test name	Result1	Result2
IgM	Negative	79.2
HBc	Negative	79.0
Ag	Negative	77.9
HBs	Negative	76.5

Test Diagnosis	Result1
HBV 2021-04	Susceptible

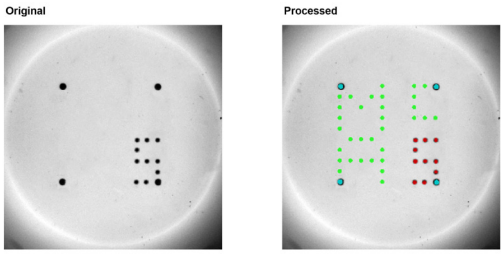
d1, Immune due to natural infection patient



Test name	Result1	Result2
IgM	Negative	77.7
HBc	Positive	488.1
Ag	Negative	77.3
HBs	Positive	501.1

Test Diagnosis	Result1
HBV 2021-04	Immune natural

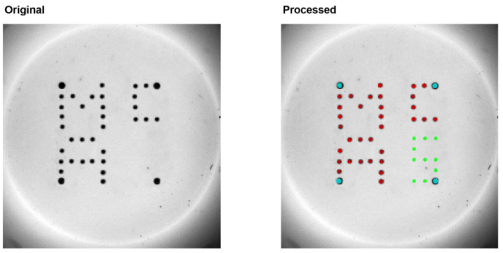
e1, Immune due to hepatitis B vaccination



Test name	Result1	Result2
IgM	Negative	78.3
HBc	Negative	74.3
Ag	Negative	77.0
HBs	Positive	503.6

Test Diagnosis	Result1
HBV 2021-04	Immune vaccine

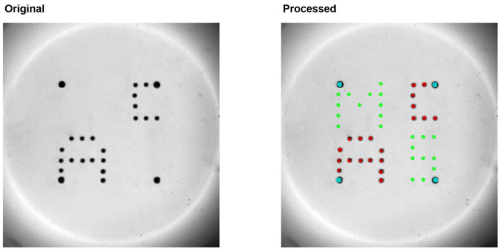
f1, Acutely infected



Test name	Result1	Result2
IgM	Positive	430.0
HBc	Positive	493.5
Ag	Positive	431.3
HBs	Negative	76.9

Test Diagnosis	Result1
HBV 2021-04	Acutely infected

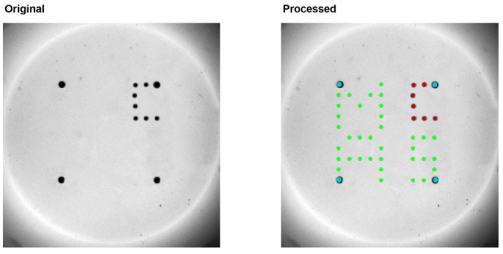
g1, Chronically infected



Test name	Result1	Result2
IgM	Negative	77.3
HBc	Positive	488.1
Ag	Positive	421.0
HBs	Negative	76.6

Test Diagnosis	Result1
HBV 2021-04	Chronically infected

h1, Interpretation unclear: Resolved or other



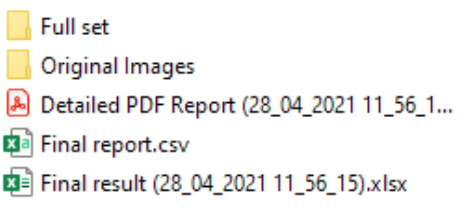
Test name	Result1	Result2
IgM	Negative	76.3
HBc	Positive	484.4
Ag	Negative	74.8
HBs	Negative	75.2

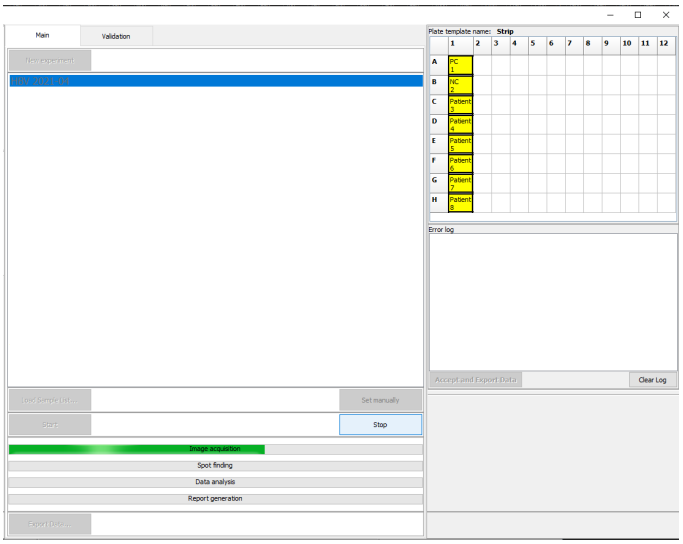
Test Diagnosis	Result1
HBV 2021-04	Resolved infection or unclear

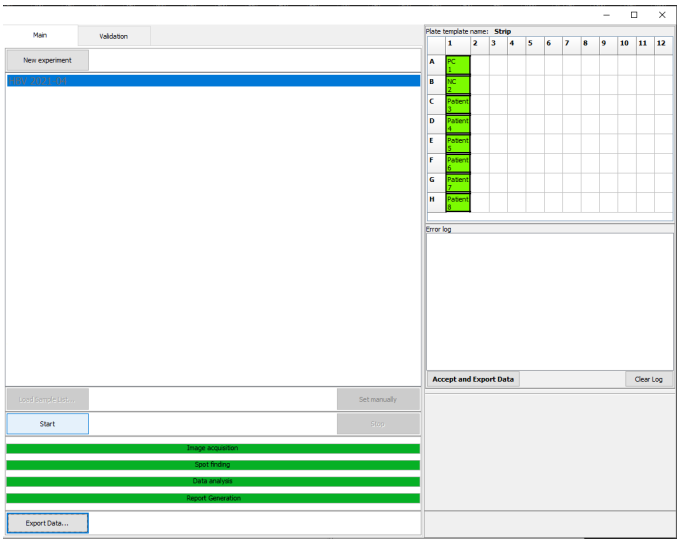
Operators mode

When the assay if fully defined, it is available in the operators module for selection. Operator is able run the assay in a few mouse clicks: load the sample ID's and press start.

Then, depending if the Autofind had no problems placing the array and the assay was set to work with out operator's visual validation, the software will export a folder consisting PDF, CSV and EXCEL reports, as well as the original images.







Conclusion

The QuantoPic software has fully satisfied the conditionals of the adapted assay. Both the R&D and operator's modes satisfied the need of the assay, as well as making it really simple to run batches of samples.

Financial Interest declaration

Author does declare financial interest by publishing this article

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Here is the list of authors who have helped with writing this article.