Uniwersytet Wrocławski

WYDZIAŁ NAUK BIOLOGICZNYCH

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Research report no ZM-2022-1 - part one conducted on 01.04.2022

1.Customer details:
Customer's name and address: Genomtec Spółka Akcyjna, Bierutowska 57-59, 51-317 Wrocław.

Number and date of the Contract: nr w rep. 139 from day 17.02.2022

2. Name and address of the Contractor: University of Wrocław, Faculty of Biological Sciences, Uniwersytecki 1 sq., 50-137 Wrocław

3. Identification of the test sample

The sample delivered by the Customer.
Mycoplasma pneumoniae FH GenBank CP002077.1

4. Measurement details
Experiment purpose: The preliminary comparative study of the Genomtec® ID Respiratory Panel 5-PLEX microfluidic reaction card and the Genomtec ID analyser for the detection of *Mycoplasma pneumoniae* from an oropharyngeal swab sample.

Start date: 25.03.2022
Finish date: 25.03.2022

Materials and Methods:

The preliminary validations comprised of two separate processes that each sample type undergo: testing on prototype-stage Genomtec ID system together with V3-prototype injection-moulded reaction card that contained all necessary liquid reagents stored in blisters: lysis buffer, washing buffer 1, washing buffer 2, elution buffer together with freeze-dried regents: binding buffer, magnetic beads and amplification reagent's targeting *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, Flu A/B, RSV and SARS-CoV-2.

Biological sample - AMPLIRUN TOTAL RESPIRATORY VIRAL PANEL CONTROL (SWAB) (VIRCELL) was dissolved according to manufacturer's instructions. 400 µl of the control material was transferred to one 1,5 ml Eppendorf tube, while remaining 100 µl was transferred to separate 1,5 ml Eppendorf tube. 400 µl of water was added to the second tube in order to obtain 5-times diluted control material. From each of the tubes 100 µl of sample was taken and added directly to dry swab (cat. No. 552C, Copan) or for the purpose of reference testing to UTM medium with swab (cat. No. 359C, Copan). Dry swab sample was then submerged in tube with transport buffer provided with Genomtec's reaction card and transferred to microfluidic cartridge using supplied disposable exact volume double-bubble pipette. Then Genomtec's proprietary protocol was conducted on the prototype of the Genomtec ID analyser.

Samples for reference testing where subject to simultaneous DNA and RNA extraction using CE-IVD approved kit - Invisorb® Spin Universal Kit (Invitek) according to manufacturer's instructions followed by Real-Time PCR reaction using CE-IVD approved kit VIASURE *C. pneumoniae*, *M.*

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pneumoniae & *L. pneumophila* Real Time PCR Detection Kit (Certest Biotec) on BioRad CFX 96 Dx Real-Time PCR thermocycler.

Sample information:
2x non-diluted sample tested on Genomtec ID system
2x 5-times diluted sample tested on Genomtec ID system

One non-diluted sample was tested using reference method using Real-Time PCR
One 5-times diluted sample was tested using reference method using Real-Time PCR

Results:
Real-Time PCR identification

Both non-diluted and 5-times diluted sample exhibited florescence signal, of C_q 31,51 and 34 respectively.

Genomtec ID system

Four tests were carried out on four separate reaction cards. Two non-diluted samples and two 5-times diluted samples have been used.

1. First 5-times diluted sample. In this case a clear increase in fluorescence is visible, starting around 16 minutes of measurement in chamber 2. No increase in fluorescence was observed in the remaining chambers (Fig. 1 and Fig. 2 in the appendix No. 2)

2. Second 5-times diluted sample. In the chambers 1, 2 and 4 an increase in the intensity of green light is visible, but in chamber 1 it is the weakest. In the chamber 6 there was an increase due to the increase in the light intensity for all wavelengths. Contrary to other increases, it is abrupt, not continuous (Fig. 3 and Fig. 4 in the appendix No. 2)

3. First non-dilluted sample. In the chambers 1, 2 and 4 there is an increase in fluorescence in each of these chambers. For chambers 1 and 2, the increase in fluorescence appeared at a similar time, between 25 and 30 minutes from the start of the measurement. In chamber 4, growth is visible for approximately 35 minutes. Readings from the remaining chambers show no increase in fluorescence (Fig 5 and Fig 6 in the appendix No. 2)

4. Second concentrated sample. For concentrated sample no. 2 the file with the measured fluorescence values was not preserved, therefore it was impossible to perform data analysis and prepare a plot of fluorescence changes. However, on the basis of the saved image of the screen during the measurement

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one can notice a clear increase in the intensity of green light in the second chamber. Growth is not visible for the remaining chambers (Fig 7 in the appendix No. 2).

Measurement was performed by: Marta Książczyk, Department of Microbiology UWr and Bartłomiej Dudek, Department of Microbiology UWr

Authorized by: Bartłomiej Dudek, Department of Microbiology UWr,
Date and signature: 01.04.22 Dudek

5. Attachments

1 The results in the form of reports from the Bio-Rad software to RT-PCR analysis are attached to the report (Attachment: report_RT-PCR 20220401 Genomtec.pdf).

2. The results in the form of reports from the Genomtec ID software are attached to the report (Attachment: report_GenomtecID 20220401 Genomtec.pdf).

6. Conclusions

Preliminary research for the tested samples reference strain *Mycoplasma pneumoniae* using the Genomtec's platform exhibited the same identification as compared to standard laboratory workflow using Real-Time PCR diagnostic assays while reducing required time to result as well as personnel and equipment needed to perform genetic testing.

Report is created by Bartłomiej Dudek Department of Microbiology UWr
Date and signature: 01.04.22 Dudek

-The end of the Report-

Wroclaw, date 01.04.2022

Fig.1

Plate Display

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D		Unk L pneumophila IC Ch pneumoniae M pneumoniae RC test 1 Reaction Cards	Unk L pneumophila IC Ch pneumoniae M pneumoniae RC test 2 Reaction Cards				Pos L pneumophila IC Ch pneumoniae M pneumoniae		NTC L pneumophila IC Ch pneumoniae M pneumoniae			
E												
F												

Fig.2

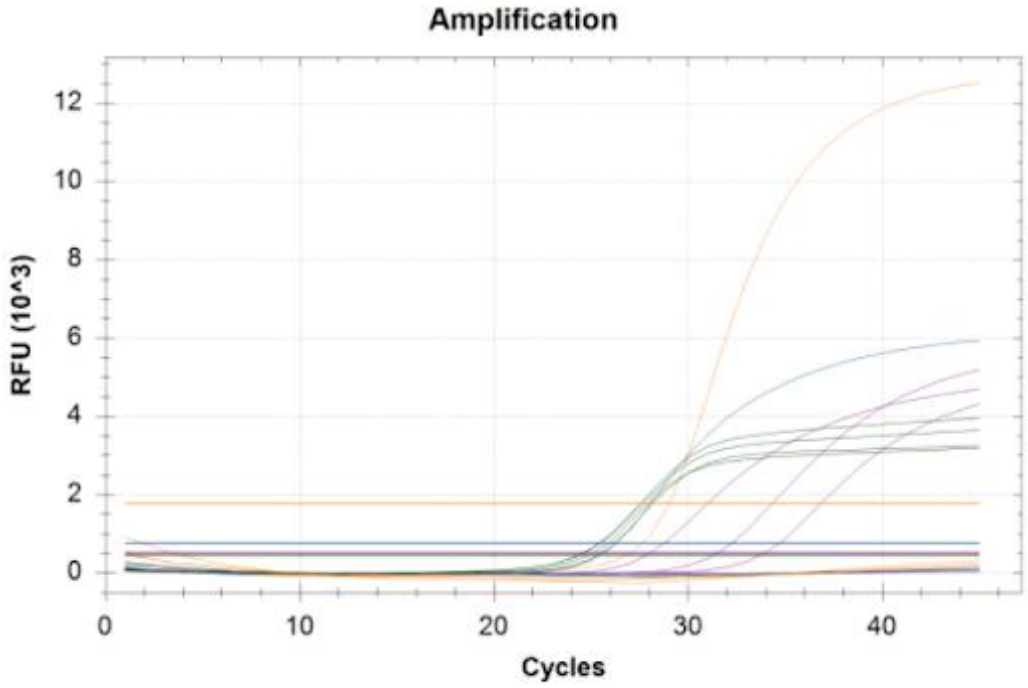

Fig.3

Quantification Data

Well	Fluor	Target	Content	Sample	Biological Set Name	Cq	Cq Mean	Cq Std. Dev
D02	Cy5	M pneumoniae	Unkn	RC test 1	Reaction Cards	31.51	31.51	0.000
D03	Cy5	M pneumoniae	Unkn	RC test 2	Reaction Cards	34.00	34.00	0.000
D07	Cy5	M pneumoniae	Pos Ctrl			28.09	28.09	0.000
D09	Cy5	M pneumoniae	NTC			N/A	0.00	0.000

Fig. 1

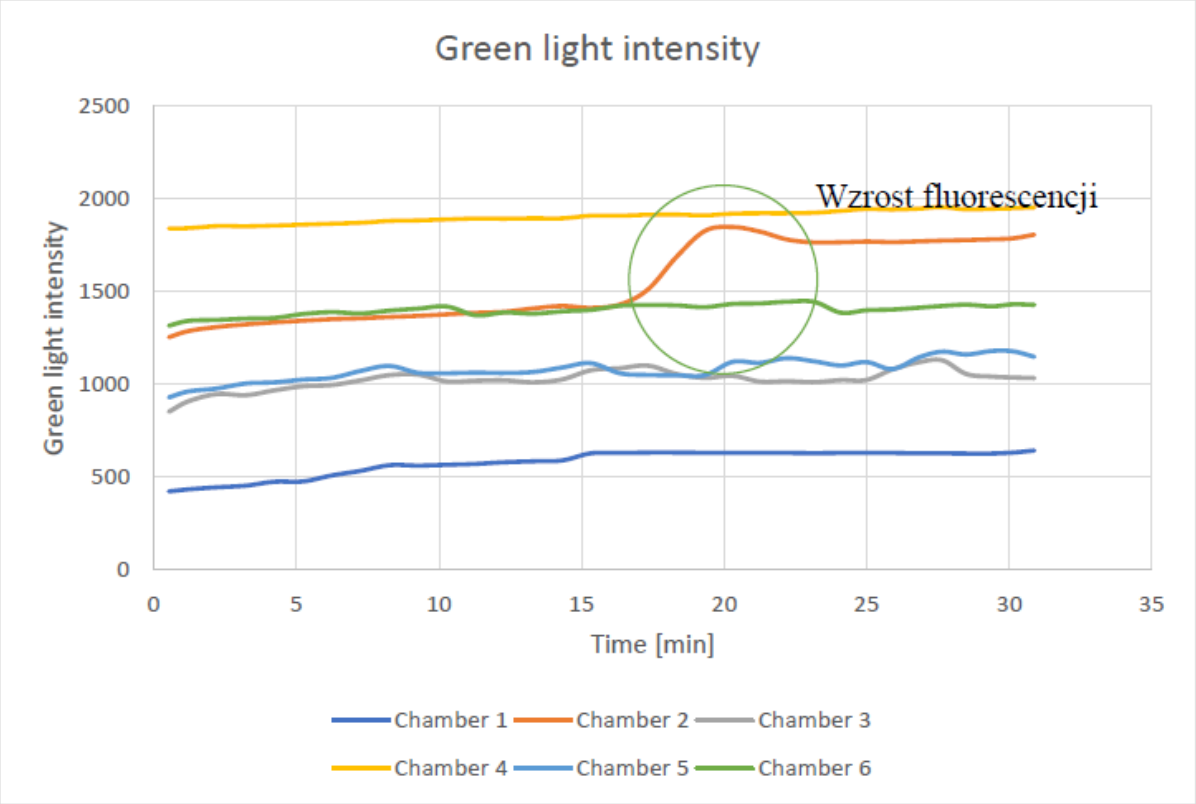
Green light intensity
Green light intensity
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
Time [min]
Wzrost fluorescencji
Chamber 1
Chamber 2
Chamber 3
Chamber 4
Chamber 5
Chamber 6

Fig. 2


GENOMTEC
Temperature ML3
PID outputs
Backlight
Fluorescence 1-2
Fluorescence 3-4
Fluorescence 5-6
Select visible:
Fluorescence reference
SET
RESET
Ref time 00:00:00
Heating time 00:30:59

Fig. 3

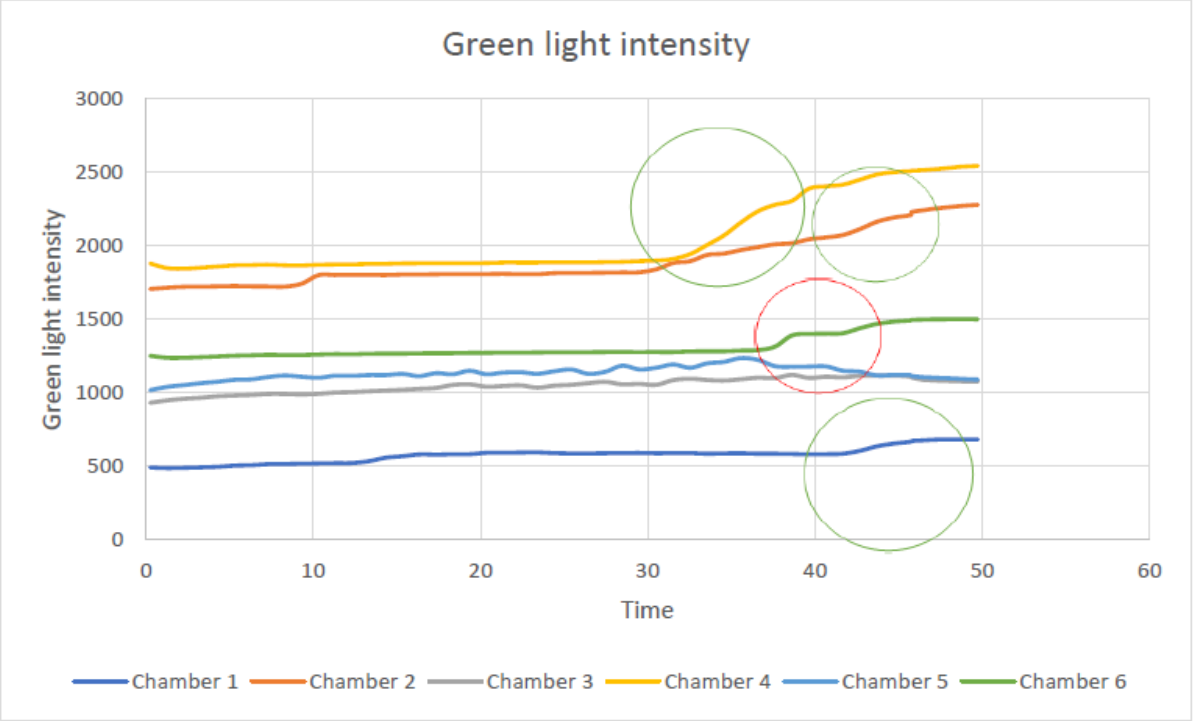
Green light intensity
Green light intensity
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
Time
Chamber 1
Chamber 2
Chamber 3
Chamber 4
Chamber 5
Chamber 6

Fig. 4


GENOMTEC
Temperature MLX
PID outputs
Backlight
Fluorescence reference
SET
RESET
Ref time 00:00:00
Heating time 00:41:53
Extraction chamber heater
Fluorescence 1-2
Fluorescence 3-4
Fluorescence 5-6
Select visible:
Set PID:
Set Emissivity:
Set Temperature:
Factor:
Fixed Pid Output

Fig. 5.

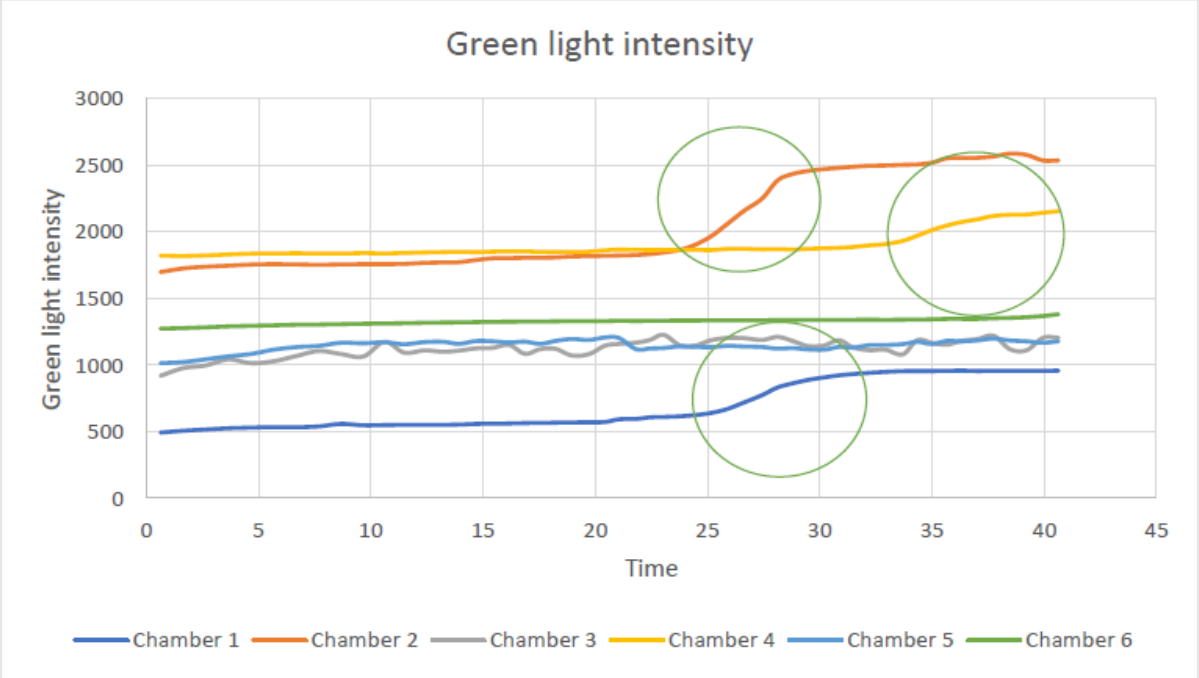
Green light intensity
Green light intensity
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
Time
Chamber 1
Chamber 2
Chamber 3
Chamber 4
Chamber 5
Chamber 6

Fig. 6


GENOMTEC
Temperature MLX
PID outputs
Backlight
Fluorescence reference
SET
RESET
Ref time
00:00:00
Heating time
00:40:26
Extraction chamber heater
Fluorescence 1-2
Fluorescence 3-4
Fluorescence 5-6
Select visible:
Set Temperature:
Fixed Pid Output

Fig. 7


GENOMTEC
Temperature MLX
PID outputs
Backlight
Fluorescence reference
SET
RESET
Ref time 00:00:00
Heating time 00:30:59
Extraction chamber heater
Fluorescence 1-2
Fluorescence 3-4
Fluorescence 5-6