Laboratory for Infectious Disease Research
Aerobiology and Immunology Core Facilities
*University of Missouri*

1020 East Campus Loop
Columbia, MO 65211
PHONE: 573-882-7195
FAX: 573-884-0791
WEB: lidr.missouri.edu

**Report for Phase II testing of the antimicrobial effect of Light Emitting Sensor (LES, Patented by Integrated Biometrics) technology fingerprint scanners on SARS-CoV-2 virus.**

Submitted to Fred Frye, Executive, Chief Scientist at Integrated Biometrics, on 30 November 2022, by Dr. Paul Anderson, PhD (Director of Infectious Diseases) and Dr. Jeffrey Whyte, PhD pursuant to the terms of agreement entered with Integrated Biometrics and the University of Missouri November 1, 2022.

**Summary:**

The University of Missouri Laboratory for Infectious Disease Research (LIDR) under contract with Integrated Biometrics, LLC of Spartanburg, SC USA employed to test the antiviral properties of the electronic field produced by a biometric fingerprint scanner utilizing Light Emitting Sensor (LES) technology.

The study utilized samples of SARS-CoV- 2 virus (SARS-Related Coronavirus 2, Isolate USA-WA1/2020, BEI Resources, NR-52281) positioned on a filter and compressed between an LES fingerprint scanner and a conductive rubber pad, then subjected to the electric field generated by the normal function of the scanner's technology. Samples were then removed and cultured on Vero E6 cells to determine the amount of surviving virus, and results confirmed by calculation of the amount inactivated by the electric field.

**Testing Process:** This process consisted of precisely placing a known quantity of virus on the IB Kojak platen. A test fixture simulating a human finger exposed the virus to the normal electric field during human fingerprint capture. The platen and simulate finger were then swabbed and samples collected. See pages 2 through 5 for a detailed description.

**Calculations:** Many calculations are used to analyze, understand, and quantify the RNA using quantitative polymerase chain reaction or qPCR tests. These are included in detail on pages 5 through 7.

**Results:** The result of this testing shows that in the capture time of normal fingerprint, 2 to 5 seconds, the electrical field eradication of virions (viable virus outside a host cell) averages **greater than 90%.** The details are shown on page 8.

# Testing Process

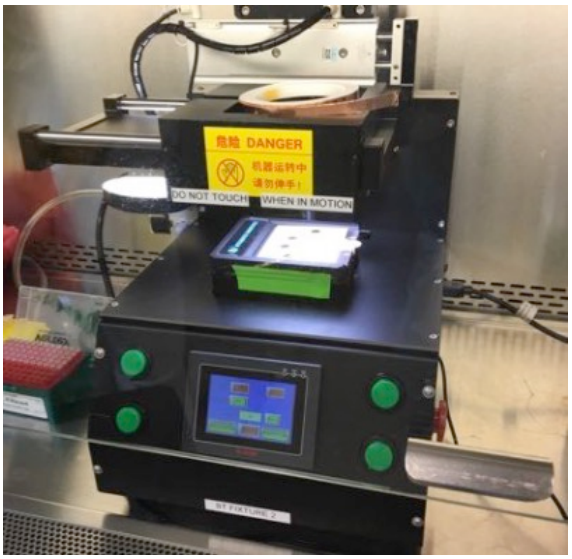
**Integrated Biometrics (IB) – SARS-CoV-2 Inactivation Vero E6 Cell Line / qPCR Viable SARS-CoV-2 Detection Assay - Date(s): 09-07-2022 – 09-08-2022**

**Investigator(s):** Microbiologist – Dr. Jeffrey Whyte, Dr. Whitney Smith, Dr. Kristina Babic and Dr. Paul Anderson

**Integrated Biometrics (IB) -** Testing of Eradication of Coronavirus (COVID-19, SARS-CoV-2) via Electric Field. Generated Fingerprint Acquisition (Phase II).

**Testing Location:** LIDR Virology Suite (BSL-3) with personal protective equipment (PPE) and powered air purifying respirator (PAPR).

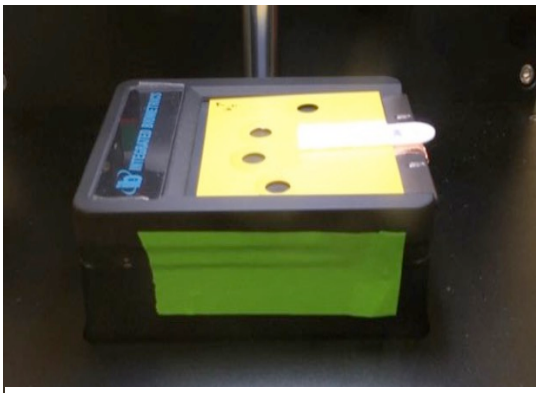
**IB Device Operator:** Tiffany Paskiewicz (Research Engineer at Integrated Biometrics)




**IB Fingerprint System ("Kojak")** situated within the biosafety cabinet (BSC; Class II, Type B2) in the BSL-3 Virology Lab.

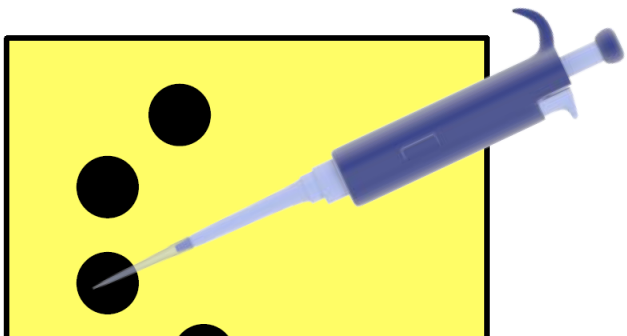
Device uses a thin 50 µm film with several layers, including a phosphor (ZnS:Cu:Cl).
An electric field across the finger's skin and the film causes the phosphor to emit light with an image of a fingerprint corresponding to the fingerprint ridges that are in contact with the top surface.

There are two configurations of the device based on the top layer:
**1. Acrylic** (**PMMA**
**2. Synthetic Polymeric Glass** (Polyhedral Oligomeric Silsesquioxane Nanocomposites, POSS)



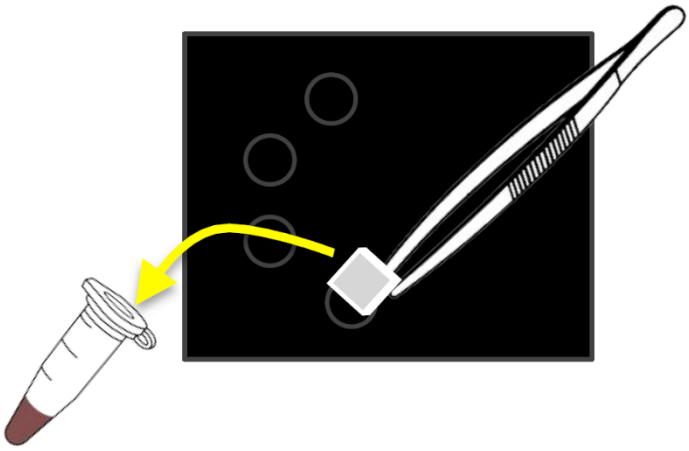
Base of **IB Fingerprint System** for application of SARS-CoV-2 stock virus solution. A LIDR-designed fingerprint template (yellow) was used to align SARS-CoV-2 stock virus solution with the platen fingerprints:

**SARS-CoV-2 Stock (T75-10)**
- LIDR propagated and characterized.
- 4.75 x $10_6$ genome equivalents/µL (GE/µL))



**Addition of SARS-CoV-2 to platen:**
• 2.5 µL virus applied per circular hole.
• 10 µL total volume (4 x 2.5 µL).

**Remove Yellow Fingerprint Template and Apply Pressure and electric field.** • Human fingerprint touch simulated by IB conductive pliable polymer pad.
• Pressure was applied to ensure even and constant pressure.

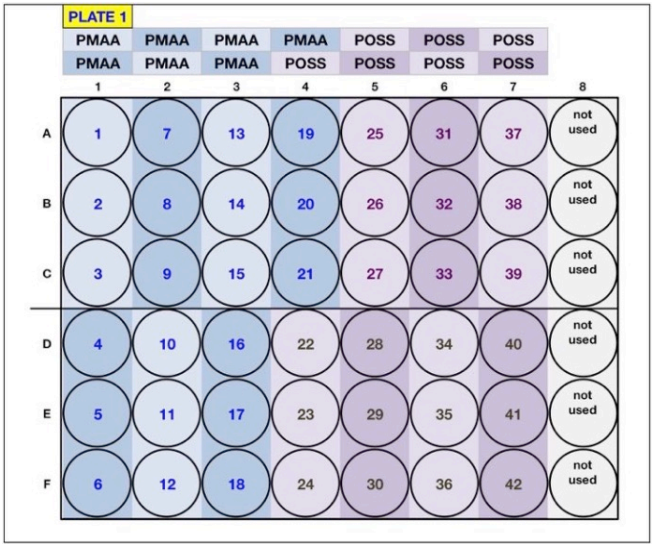


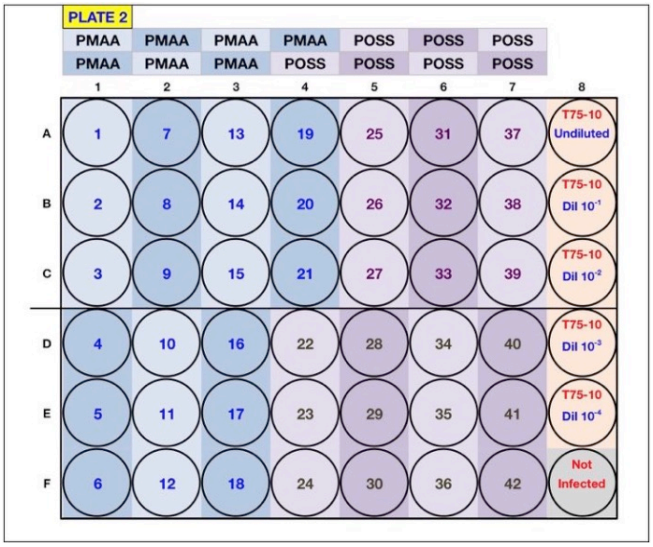
**Collection Of Virus from Platen After Voltage Application**
• Platen surface (**top** and **bottom**) swabbed with 1 $cm^2$ sterile gauze.
• Gauze placed in tube with 500 µL cell culture medium (0% FBS).
• Tubes frozen at -80°C until Viability Assay.
• Platen surface sterilized with Clorox wipe between tests.

➢ **Day 1**: **Preparation of Vero E6 Cells for SARS-CoV-2 Exposure (Immunology Lab - BSL-2):**
• Seed **Vero E6** in two 48-well plates (30,000 cells/well).
• 250 µL/well growth medium (DMEM +10% FBS +1 mM GlutaMax +10 µg/mL gentamicin).
• Incubate cells for 24 hours (37°C, 5% $CO_2$, humidified environment) until 80-90% confluent.

➢ **Day 2: Vero E6 Infection with SARS-CoV-2 Samples Treated with IB Devices (Virology Lab - BSL-3):**

• Vero E6 exposed (60 min) to duplicate (100 µL/well) of **IB Device-Treated** samples or a SARS-CoV-2 stock dilution series (**T75-10**; propagated and characterized at LIDR, 4.69E+06 GE/µL).




| Sample ID | Configuration |
|---|---|
| 1 | PMMA, 2 sec |
| 2 | PMMA, 2 sec |
| 3 | PMMA, 2 sec |
| | |
| 4 | PMMA, 5 sec |
| 5 | PMMA, 5 sec |
| 6 | PMMA, 5 sec |
| | |
| 7 | PMMA, 10 sec |
| 8 | PMMA, 10 sec |
| 9 | PMMA, 10 sec |
| | |
| 10 | PMMA, 15 sec |
| 11 | PMMA, 15 sec |
| 12 | PMMA, 15 sec |
| | |
| 13 | PMMA, 30 sec |
| 14 | PMMA, 30 sec |
| 15 | PMMA, 30 sec |
| | |
| 16 | PMMA, 60 sec |
| 17 | PMMA, 60 sec |
| 18 | PMMA, 60 sec |
| | |
| 19 | PMMA, Control |
| 20 | PMMA, Control |
| 21 | PMMA, Control |

| Sample ID | Configuration |
|---|---|
| 22 | POSS, 2 sec |
| 23 | POSS, 2 sec |
| 24 | POSS, 2 sec |
| | |
| 25 | POSS, 5 sec |
| 26 | POSS, 5 sec |
| 27 | POSS, 5 sec |
| | |
| 28 | POSS, 10 sec |
| 29 | POSS, 10 sec |
| 30 | POSS, 10 sec |
| | |
| 31 | POSS, 15 sec |
| 32 | POSS, 15 sec |
| 33 | POSS, 15 sec |
| | |
| 34 | POSS, 30 sec |
| 35 | POSS, 30 sec |
| 36 | POSS, 30 sec |
| | |
| 37 | POSS, 60 sec |
| 38 | POSS, 60 sec |
| 39 | POSS, 60 sec |
| | |
| 40 | POSS, Control |
| 41 | POSS, Control |
| 42 | POSS, Control |

- After 60 min, added 250 µL/well of complete growth medium.
- **Time 0 hr Sample:** Immediately collected 100 µL aliquot from each well and stored at -80°C.
- Incubated cells incubated for **40 hours** (37°C, 5% $CO_2$, humidified environment).

➢ **Day 4: Time 40 hr sample collection for RNA isolation and qPCR analysis for SARS-CoV-2 (Virology – BSL-3 and Immunology Lab – BSL-2):**

• **Time 40 hr Sample:** Collected 100 µL aliquot from each well and stored at -80°C.
• Isolate RNA from **Time 0 hr** and **Time 40 hr** samples (Virology BSL-3) and qPCR.
• SARS-CoV-2 quantified as genome equivalents of virus per mL of growth medium (GE/mL).
• Effectiveness of **IB device configurations** to inactivate SARS-CoV-2 was determined as **Log Reduction** and **Percent Reduction** of virus (GE/mL).

## SARS-CoV-2 RNA Isolation from Vero E6 Cell Medium and Quantitative PCR (qPCR)

### SARS-CoV-2 viral RNA isolation from samples (Kingfisher Flex)

• SARS-CoV-2 viral RNA isolated from **100 µL Vero E6 medium**.
• Final RNA isolate volume = **90 µL**
• Viral RNA isolates are stored at -80°C (Lab 132 Freezer).

### SARS-CoV-2 detection by qPCR (ABI 7500 )

• **10 µL** of viral RNA analyzed by qPCR to detect SARS-CoV-2.
• TaqPath SARS-CoV-2 detection assay (**S gene** fragment).
• Each successive qPCR **cycle threshold** (**Ct**) value = one doubling of viral RNA.

## Calculations

### Calculation of SARS-CoV-2 Concentrations in IB Samples

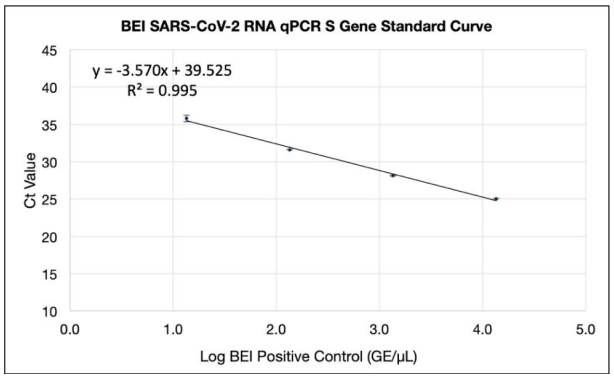
• A SARS-CoV-2 RNA stock (BEI Corporation) is used to generate a qPCR standard curve of **Ct** versus **SARS-CoV-2** (GE/µL).

• The regression equation (slope and intercept) is used to calculate SARS-CoV-2 concentrations (GE/µL) in each **IB** qPCR sample.

| BEI qPCR Standard Dilution Tube ID | Pos Ctrl GE/µL | Log Pos Ctrl GE/µL | Ct Value | Ct StdDev |
|---|---|---|---|---|
| Dilution 4 | 13.5 | 1.1 | 35.8 | 0.4 |
| Dilution 3 | 135 | 2.1 | 31.6 | 0.0 |
| Dilution 2 | 1,350 | 3.1 | 28.1 | 0.1 |
| Dilution 1 | 13,500 | 4.1 | 25.0 | 0.0 |

* GE = Genome Equivalents. Current BEI postive control (Product #NR-52347) lot number 70044886.

That stock vial contains 1.35 x $10^8$ GE/mL. 10 µL of each dilution is added per PCR reaction well.



## Example Calculation of SARS-CoV-2 Concentration in IB Sample (POSS Control)

From BEI SARS-CoV-2 Standard Curve: ***slope*** = -3.570 ***intercept*** = 39.525

**POSS Control** SARS-CoV-2 (log GE/µL) = $\frac{\textit{Sample Ct} - \textit{intercept}}{\textit{slope}}$

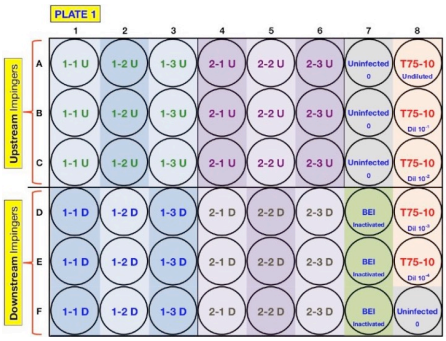
**POSS Control**
SARS-CoV-2 (log GE/µL) = $\frac{27.20 - 39.525}{-3.570}$ = **3.454** GE/µL (log) → $10^{3.454}$ = **2,842** GE/µL *

* Values rounded for display

| IB Configuration | IB Rep | Vero E6 Rep | Collection Time | Ct Value | GE/µL Log | GE/µL SARS-CoV-2 |
|---|---|---|---|---|---|---|
| POSS, Control | 1 | 1 | 0 hr | **27.20** | 3.454 | 2,842 |
| POSS, Control | 1 | 2 | 0 hr | **27.15** | 3.466 | 2,925 |
| POSS, Control | 2 | 1 | 0 hr | **27.19** | 3.456 | 2,858 |
| POSS, Control | 2 | 2 | 0 hr | **27.30** | 3.424 | 2,655 |
| POSS, Control | 3 | 1 | 0 hr | **27.31** | 3.421 | 2,635 |
| POSS, Control | 3 | 2 | 0 hr | **27.23** | 3.445 | 2,786 |

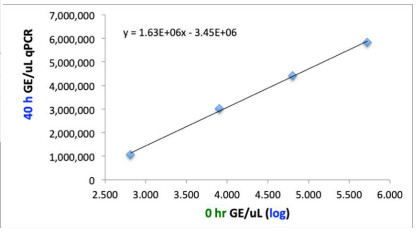
## Assay Principle: Calculation of 0 hr *Calculated* SARS-CoV-2 concentrations (GE/µL)

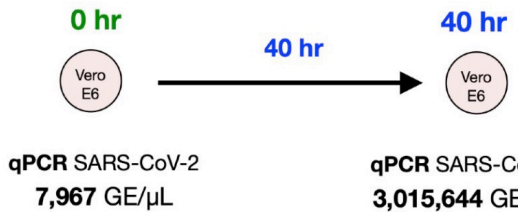
**Example of SARS-CoV-2 propagation in untreated T75-10 Dilution 2 ($10^{-2}$) at 0 hr and 40 hr (qPCR GE/µL)**




| T75-10 SARS-CoV-2 Stock in Vero E6 | 0 hr GE/µL (qPCR) | 0 hr GE/µL (log qPCR) | 40 hr GE/µL (qPCR) |
|---|---|---|---|
| T75-10 10^-3 | 647 | 2.811 | 1,060,778 |
| T75-10 10^-2 | 7,967 | 3.901 | 3,015,644 |
| T75-10 10^-1 | 63,314 | 4.802 | 4,406,996 |
| T75-10 Stock | 527,548 | 5.722 | 5,824,566 |

* T75-10 $10^{-4}$ below detection




**qPCR** SARS-CoV-2
**7,967** GE/µL

**qPCR** SARS-CoV-2
**3,015,644** GE/µL

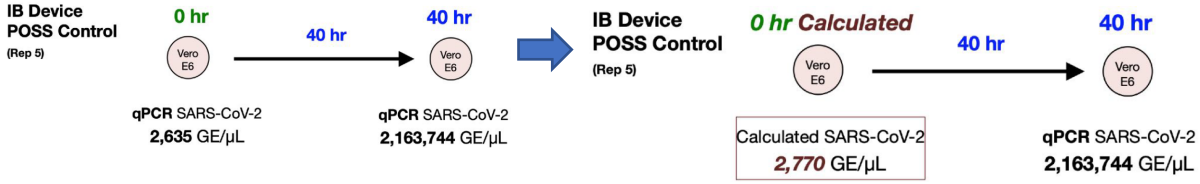
**0 hr *Calculated* (log GE/µL)** = $\frac{\textbf{40 hr} \text{ qPCR (GE/µL)} - \textit{intercept}}{\textit{slope}}$

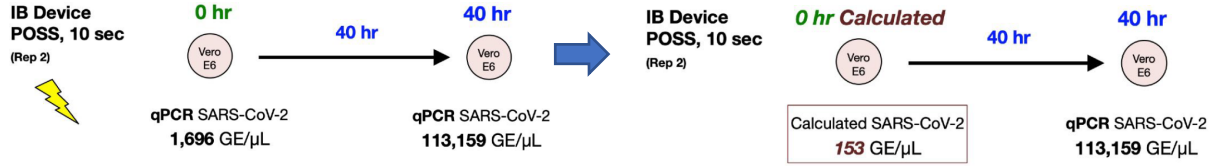
# Calculating viral eradication after exposure to IB's LES Film electric field

**0 hr and 40 hr mean qPCR Measured (GE/µL)**

**0 hr *Calculated* mean GE/µL from 40 hr mean qPCR Measured (GE/µL)**

* Assuming no inactivation treatment applied




**Assessment of Viable SARS-CoV-2 Results (0 hr *Calculated*)**

**0 hr *Log Reduction* = *log* (0 hr Measured / 0 hr *Calculated*)**

$$\text{0 hr } \textit{Percent Reduction} = \frac{(\text{0 hr Measured} - \text{0 hr } \textit{Calculated})}{\text{0 hr Measured}}$$

**1** log reduction = **90%** reduction
**2** log reduction = **99%** reduction
**3** log reduction = **99.9%** reduction
**4** log reduction = **99.99%** reduction
**5** log reduction = **99.999%** reduction
**6** log reduction = **99.9999%** reduction

# Results

**Table 1:** IB Device SARS-CoV-2 Inactivation. Mean **Measured 0 hr**, Mean **Measured 40 hr,** and Mean ***Calculated* 0 hr** SARS-CoV-2 concentrations (GE/µL) with Log Reduction and Percent reduction.

| **Integrated Biometrics Configuration** | **Measured** 0 hr Mean SARS-CoV-2 by **qPCR** (GE/µL) | **Measured** 40 hr Mean SARS-CoV-2 by **qPCR** (GE/µL) | *Calculated* 0 hr Mean SARS-CoV-2 **(GE/µL)** | **Vero E6 and qPCR Replicates** | **Percent Reduction** (0 hr *Calculated*) | **Log Reduction** (0 hr *Calculated)* |
|---|---|---|---|---|---|---|
| **PMMA,** Control | 2,359 | 854,583 | *447* | 2 | 81.1% | 0.72 |
| **PMMA,** 2 sec | 2,701 | 594,974 | *349* | 2 | 87.1% | 0.89 |
| **PMMA,** 5 sec | 2,719 | 57,712 | *142* | 3 | 94.8% | 1.28 |
| **PMMA,** 10 sec | 2,173 | 389,294 | *227* | 2 | 89.6% | 0.98 |
| **PMMA,** 15 sec | 1,453 | 119,328 | *155* | 2 | 89.3% | 0.97 |
| **PMMA,** 30 sec | 1,814 | 272,573 | *192* | 1 | 89.4% | 0.98 |
| **PMMA,** 60 sec | 1,794 | 88,585 | *148* | 3 | 91.7% | 1.08 |
| **POSS,** Control | 2,783 | 1,299,363 | *1,049* | 6 | 62.3% | 0.42 |
| **POSS,** 2 sec | 2,721 | 354,226 | *261* | 6 | 90.4% | 1.02 |
| **POSS,** 5 sec | 1,560 | 177,887 | *178* | 4 | 88.6% | 0.94 |
| **POSS,** 10 sec | 1,714 | 163,328 | *165* | 4 | 90.4% | 1.02 |
| **POSS,** 15 sec | 2,739 | 176,849 | *170* | 3 | 93.8% | 1.21 |
| **POSS,** 30 sec | 2,182 | 83,173 | *147* | 4 | 93.3% | 1.17 |
| **POSS,** 60 sec | 2,091 | 80,545 | *147* | 2 | 93.0% | 1.15 |