

**Evaluation of IM/Q610/419-01A-18 Silverlab Healthcare  
sample effectiveness against SARS-CoV-2 in an *in  
vitro* cell based assay**

**CSIR NextGen Health  
Array Technology and Companion Diagnostic Group**

## CSIR BIOSCIENCES

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### FINAL TECHNICAL REPORT

SPONSOR: Silverlab Healthcare

TESTING FACILITY: CSIR NextGen Health cluster,  
Array Technology Laboratory

STUDY CONDUCTED BY: Dr. Kabamba Alexandre



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July 2021

## 1. BACKGROUND

In late 2019 several individuals were hospitalized in Wuhan in China with what appeared to be cases of severe respiratory illnesses (Kannan *et al.*, 2020). The disease was called the 2019 coronavirus disease (COVID-19) and the etiological agent was the coronavirus-2 or SARS-CoV-2 (Nie *et al.*, 2020; van Doremalen *et al.*, 2020). One of the main routes of transmission of this virus is through contaminated aerosols resulting from coughing or sneezing for example (Kannan *et al.*, 2020). The virus incubation time in an infected individual is 2 to 14 days. However, even asymptomatic infected people can effectively spread SARS-CoV-2. Most COVID-19 cases or 80% is either asymptomatic or have only mild symptoms. However, 20% of all those infected will develop severe illness with the mortality rate standing at about 5% across the globe. So far globally there are over 188 million individuals infected with SARS-CoV-2 and nearly 4.05 million deaths. Early 2020 the World Health Organization (WHO) declared COVID-19 a global pandemic. COVID-19 has affected millions of people and caused many deaths in the world, most developed and industrialized countries; as well as taking many economies into recessions or at the brink of collapse. However, although many of these developed countries are claiming to be closer to passing the peak of the spread of the virus, the same cannot be said of developing countries, which constitute the majority in the world. Since the start of the pandemic, scientists around the world instigated research in the quest to finding vaccines that can be used to regulate and prevent the spread of the virus. Late 2020 and early 2021, several vaccines were approved, namely, Johnson & Johnson, Pfizer and AstraZeneca vaccines to name a few, which are currently being administered around the world as a way of controlling the virus.

Other ways to prevent SARS-CoV-2 infection is by disinfecting all exposed surfaces with detergents, sanitizers, soaps and so on. However, there are many products in the market that are claimed to be active against SARS-CoV-2 without any proof of such. It is in this

context that Silverlab Healthcare contacted the CSIR with IM/Q610/419-01A-18 for evaluation against SARS-CoV-2.

The virus used in the evaluation was generated in 293-T cells by co-transfection of a construct carrying the SARS-CoV-2 envelope and a plasmid encoding the luciferase reporter gene. This was followed by the determination of the virus tissue culture infectious dose 50 (TCID<sub>50</sub>) before treatment with IM/Q610/419-01A-18.

In summary:

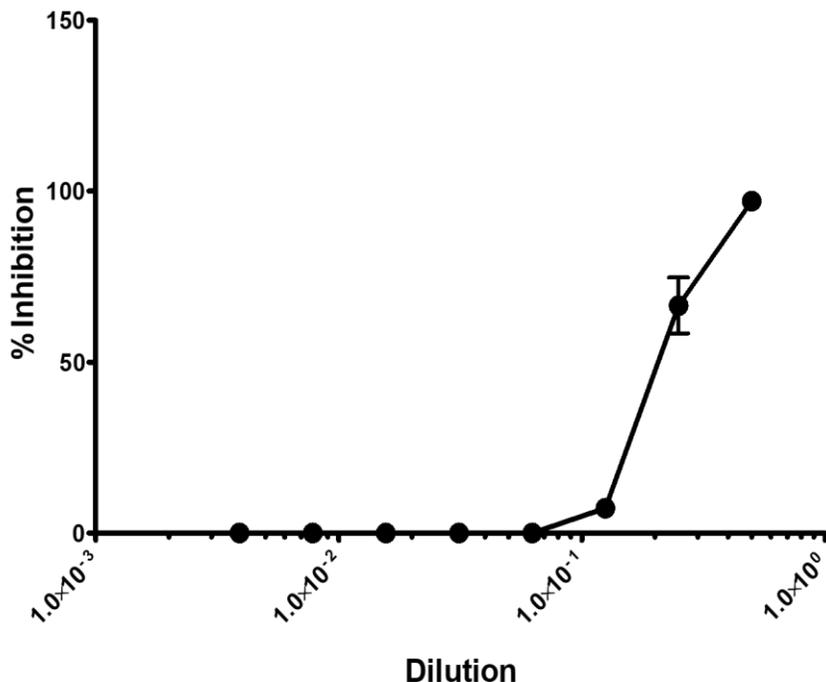
- Against SARS-CoV-2, the IM/Q610/419-01A-18 sample had an ID<sub>50</sub> value of 5, see Table 1. The value was determined after plotting the percentage inhibition against the dilution (Figure 1). Furthermore, the TC<sub>50</sub> value was greater than the dilution we performed the assay with (1 in 2 dilution) (Figure 2). Thus, since in all cases IM/Q610/419-01A-18 almost did not show signs of toxicity to the target cells, the accurate TI could not be determined. However, judging from Figure 2, we expect the TI to be high for IM/Q610/419-01A-18.

**Table 1:** IM/Q610/419-01A-18 sample ID<sub>50</sub>, TC<sub>50</sub> and TI values against SARS-CoV-2

Sample	<sup>a</sup> ID <sub>50</sub>	TC <sub>50</sub>	TI
	SARS-CoV- 2		SARS-CoV-2
<b>IM/Q610/419-01A-18</b>	5	>2	3

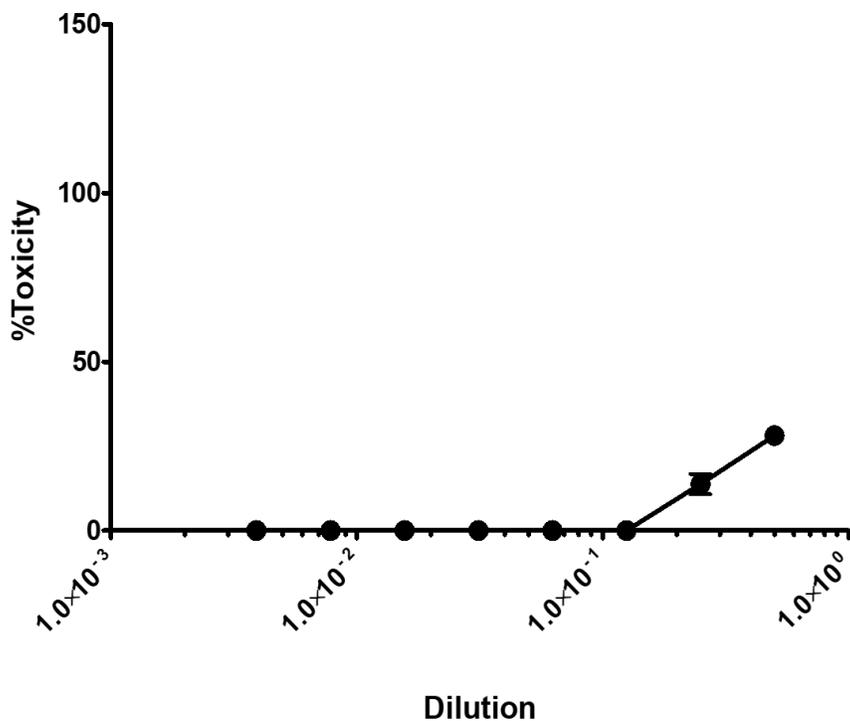
<sup>a</sup> The inverse of the dilution i.e. 1/dilution

SARS-CoV-2 IM/Q610/419-01A-18



**Figure 1: IM/Q610/419-01A-18 inhibition of SARS-CoV-2.** The virus was incubated with IM/Q610/419-01A-18 before infection of 293T-ACE cells. After 72 hours incubation the inhibition of infection was determined by measuring luminescence. Data shown represent the results of three independent experiments, and bars represent the means  $\pm$  standard deviation

IM/Q610/419-01A-18



**Figure 2: IM/Q610/419-01A-18 toxicity evaluation.** The sample was incubated with 293T-ACE cells followed by the determination of cytotoxicity using the MTT assay. Data shown are means  $\pm$  standard deviation of three independent experiments.

## 2. Materials

- Cell lines: 293T-ACE cells (American Type Culture Collection) and 293-T cells (American Type Culture Collection)
- Cell culture growth media (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal bovine serum and penicillin/streptomycin)
- Other cell culture components: Phosphate Buffered Saline (PBS), Trypsin-EDTA (0.25% trypsin, 1mM EDTA), DEAE dextran,
- Virus growth components: SARS-CoV-2 pseudoviruses made from a plasmid encoding SARS-CoV-2 SA strain envelope respectively and a plasmid encoding the luciferase reporter gene (pNL4-3.luc.RE)
- Plasmid transfection reagent: X-tremeGENE,
- Luminescence reading reagent: Luciferase substrate (Bright Glo™ Reagent),
- Silverlab Healthcare sample (IM/Q610/419-01A-18).
- Luminometer

## 3. Laboratory equipment and consumables

- CO<sub>2</sub> incubator
- Biosafety cabinet
- Bench top centrifuge with an adaptor for 96 well plates
- Water bath
- Flat bottom 96 well plates
- Flat bottom 96 well black plates
- T75 tissue culture flasks

## 4. Method

### 4.1 Generation of SARV-CoV-2 pseudoviruses

SARS-CoV-2 viruses were generated by co-transfection of the Envelope containing plasmid, with a plasmid carrying the luciferase reporter gene (Wei *et al.*, 2003), into  $2 \times 10^6$  293T cells/10ml of growth media using the X-tremeGENE transfection reagent (Sigma Aldrich, Missouri, US) transfection reagent (Roche Applied Science, Indianapolis, IN). The TCID<sub>50</sub> of the virus stock was quantified by infecting 293T-ACE cells with serial 4-fold dilution of the supernatant in quadruplicate in the presence of DEAE dextran (37.5 µg/mL) (Sigma-Aldrich, St. Louis, MO). The Bright Glo™ Reagent (Promega, Madison, WI) was used to measure infection after 72 h of tissue culture, according to the manufacturer's instructions. Luminescence was measured in the luminometer Tecan Infinite F500.

### 4.2 Assay set up to investigate IM/Q610/419-01A-18 inhibition of SARS-CoV-2 infection of 293T-ACE cells

A 96 well plate was used and the experiment was done in triplicates. Cell control as well as virus control wells were included. A 2-fold serial dilution was performed by adding 100 µl of IM/Q610/419-01A-18 to 100 µl of growth media. After the dilution series, 50 µl of SARS-CoV-2 was added to all wells except the cell control wells. This was followed by incubation at 37°C for 1 hour to allow sample to interact with the virus. Afterwards, 10 000 cells/100 µl/well of 293T-ACE cells were added and centrifuged at 3500 rpm for 3 hours and 30 minutes. After centrifugation, the cells were incubated at 37°C, 5 % CO<sub>2</sub> and 95 % humidity for 72 hours. The incubation period was followed by the removal of 150 µl of growth media and the addition of 100 µl of Bright-Glo luciferase substrate to all the wells. Then the cells were incubated for 2 minutes at room temperature. Afterwards 150 µl was transferred to the corresponding wells of a 96-well black plate and luminescence was read using luminometer Tecan Infinite F500.

### 4.3 Cytotoxicity assay for IM/Q610/419-01A-18 in 293T-ACE cells

The cytotoxicity assay for the IM/Q610/419-01A-18 sample was performed by first seeding 10 000 cells/well/100 µl of 293T-ACE cells in a 96 well plate for 24 hours at 37°C, 5 % CO<sub>2</sub> and 95 % humidity. After 24 hours, a 2-fold serial dilution of the sample was performed and 100 µl was transferred to the plate containing cells, except in the cell control wells. The cells were then incubated for 72 hours. Following the 72 hours incubation media was removed and replaced with 25 µl of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (5 mg/ml) (Sigma-Aldrich, St. Louis, MO). This was followed by 3 hours incubation at 37°C to allow the formazan product to form from viable cells. After incubation, the MTT was removed and 100 µl DMSO was added and incubated for 15 minutes at room temperature. Afterwards the absorbance was read at a wavelength of 620 nm using the Tecan Infinite F500 luminometer.

## 5. Conclusion

The IM/Q610/419-01A-18 Silverlab Healthcare sample showed activity of 97.1 and 66.6% *in vitro* at dilutions of  $\frac{1}{2}$  and  $\frac{1}{4}$  against SARS-CoV-2, respectively. The  $ID_{50}$  of the sample was 5 against SARS-CoV-2. In addition, the sample did not show cytotoxicity to 293T-ACE cells used in the study as target cells, as shown in Figure 2. Based on this, it is possible that the sample TI is high, which is desirable for drug candidates. Thus, IM/Q610/419-01A-18 Silverlab Healthcare sample tested in this study can be declared active against SARS-CoV-2 *in vitro*.

## 6. REFERENCES

Schmidtke M., Schnittler U., Jahn B., Dahse H.M., Stelzner A., 2001. A rapid assay for evaluation of antiviral activity against coxsackie virus B3, influenza virus A, and herpes simplex virus type 1. *Journal of Virological Methods*, **95**:133–143.

## CSIR GENERAL TERMS AND CONDITIONS

*In these general contract conditions, "CSIR" means The CSIR, a juristic person, established in accordance with the Scientific Research Council Act, Act No. 46 of 1988.*

### 1. PURPOSE OF THE PROPOSAL

- 1.1 The purpose for the request of the work as set out in the accompanying Proposal must be disclosed to the satisfaction of the CSIR, and the CSIR is hereby authorized by the client to make whatever enquiries it deems fit to establish such purpose.
- 1.2 The contents of the Proposal are confidential, and shall not be divulged to any unauthorized third party.
- 1.3 Any reference hereto to "Contract" shall mean the Proposal as accepted by the Client, together with these general conditions of contract.

### 2. GOODS AND SERVICES

- 2.1 Services shall be rendered, subject to the client's acceptance of:
  - i. the scope of the work, and, where applicable, the procedure to be followed, as set out in the Proposal;
  - ii. the proposed duration and date of commencement of the work;
  - iii. the agreed price and conditions of payment;
  - iv. all other conditions contained in the attached written Proposal.
- 2.2 Any materials, apparatus or equipment delivered by or on behalf of the client to the CSIR, or to the premises of a sub-contractor of the CSIR, after acceptance of the Proposal, shall be accepted, retained and used at the client's risk. The client will be responsible to take out the necessary Insurance cover applicable to the situation.
- 2.3 Unless otherwise stated in the Proposal or the parties agree otherwise in writing, the CSIR shall have no obligation to commence the carrying out of the work or services before all materials or goods in the agreed form and numbers have been placed at CSIR's disposal.
- 2.4 The prices are based on the prevailing cost of material and labour and are subject to alteration should there be any changes in any of the following factors or circumstances subsequent to the date of this Proposal, which may have an effect on the price at the time of delivery:
  - i. exchange rates;
  - ii. price of goods quoted or of any part of the work sub-contracted to a third party and import duty, freight and cartage.
- 2.5 The period and terms for the replacement of defective parts or goods are limited to the period and terms of the express guarantee contained in the Proposal, and under no circumstances will any claim be entertained for any consequential damage or loss of any kind whatsoever.
- 2.6 Unless otherwise stated in the Proposal, the place of delivery of the goods is the CSIR's main campus in Pretoria, and all costs of delivery to the Client's premises will be for the account of the Client.
- 2.7 Ownership of the goods, all equipment, component, spares and materials will remain with the CSIR until such time as the full contract price has been paid

to the CSIR. The Client accepts the sole risk and responsibility for the goods from date of delivery.

- 2.8 The Client shall not be entitled to reduce the contract price, or contract amount, or scope of work stated in the Proposal without the CSIR's prior written consent. If the Client reduces the scope of work or the goods ordered, the Client shall still remain liable for the full contract price, unless the CSIR agrees otherwise in writing.

### 3. REPORTS

- 3.1 The contents of any interim reports issued by the CSIR are confidential unless the CSIR and the client agree to disclose such report. No reference may be made to the CSIR or any of its operating units or centres, or employees in any marketing materials, or for any other purpose whatsoever without the CSIR's prior written consent.
- 3.2 The final report will, subject to Clause 6 below, be the property of the client and may be published by it, provided that the CSIR shall be indemnified by the Client against any claims for damages that may result from the publication of that report.
- 3.3 The CSIR shall not publish any results without the Client's consent. CSIR shall be entitled to use the technical information derived from the work, and to publish same, but shall not to identify the Client or the investigation in doing so, without the prior approval of the Client.

### 4. ACCEPTANCE OF CONDITIONS

Acceptance of the Proposal shall be deemed to include acceptance of the Conditions contained herein, and the person accepting the Proposal on behalf of the Client, where applicable, warrants that he/she is duly authorized so to act on behalf of the Client and also warrants that the legal entity of the Client is as stipulated in the Proposal. Should it subsequently appear that he/she was not in fact properly authorized or that incorrect information was supplied with regard to the Client's legal status, he/she will be liable as surety and co-principal debtor, in his/her personal capacity, as against the CSIR, for the fulfillment of all the obligations contained herein, and will in his/her personal capacity be bound by all the terms and conditions contained in both the Proposal and herein.

### 5. PAYMENT

- 5.1 If the attached Proposal stipulates an advance payment, the CSIR will not commence work as set out in the Proposal until the said advance payment has been received by the CSIR. Any delay occasioned by a late payment, shall be added to the contract period.
- 5.2 All other payments will be strictly net within 30 (THIRTY) days of the date of invoice, or the date on which payment is due to the CSIR, in terms of the Proposal. Any amount not paid on due date shall bear interest at a rate of 2 % (TWO PERCENT) above the prime overdraft rate (per annum) charged by ABSA Bank from time to time, calculated and compounded daily in advance as from the due date.
- 5.3 All payments shall be made without deduction or set-off of whatsoever nature and no discounts for early settlement will be allowed on the amounts due to CSIR.

5.4 Should the Client fail to pay any instalment punctually on due date or commit a breach of any of the provisions of this contract, the CSIR shall be entitled to forthwith claim payment of the full outstanding balance of the contract price without any notice to the Client, as well as all legal costs incurred on the scale as between attorney and own client, including collection commission.

#### **6. INTELLECTUAL PROPERTY**

6.1 It is recorded that any Intellectual Property, created prior to the date of acceptance of the attached Proposal, shall vest exclusively with the party/parties who at that stage owned the same.

6.2 Intellectual property that may arise from the work shall vest in CSIR. No agreement in respect of the transfer of Intellectual Property shall be of any force and effect, unless reduced to writing and signed by both parties.

6.3 For purposes of this clause "Intellectual Property" means: the patents, designs, know-how, copyright and trade marks which relate to the goods or services; whilst:-

- i. the "know-how" means all confidential information of whatever nature relating to the Intellectual Property and its exploitation as well as all other confidential information generally relating to the manufacture, use and sale of the goods including technical information, manufacturing technique and designs, specifications, formulae, systems, processes, information concerning materials and marketing and business information generally;
- ii. "the patents" means the registered patents and patent applications;
- iii. "the trade marks" means the registered trade marks and trade mark applications;
- iv. "the designs" means the registered designs and design applications, and any other registerable inventions/trade or brand names and designs;
- v. "copyright" means copyright in computer software programmes, computer data bases, data messages, and reports.

#### **7. CONFIDENTIALITY**

7.1 For purposes of this clause, "Confidential Information" means information that (i) relates to the Disclosing Party's past, present or future research, development, business activities, products services and technical knowledge relating to the work, and (ii) either has been identified in writing as confidential, or is of such a nature, or has been disclosed in such a way that it is obvious to the other party that it is claimed as confidential. The party disclosing the confidential information is referred to as "the Disclosing Party" and the party receiving such information as "the Recipient".

7.2 The parties shall:

- i. treat as strictly confidential and secret any and all information given or made known to them during the contract period.
- ii. keep all Confidential information secret towards third parties and only use it in co-operation with each other for the purpose expressly agreed upon by the Parties and to disclose same to their employees only on the basis of the need to know;
- iii. accept responsibility for the observance by their employees of the secrecy undertakings contained herein;

7.3 The above undertakings shall not apply to:

- i. Information which at the time of disclosure is published or otherwise generally available to the public;
- ii. Information which the Recipient can show was in its possession at the time of disclosure by the Disclosing Party;
- iii. Information rightfully acquired from others who did not obtain it under pledge of secrecy to either of the parties;
- iv. Information contained in any final report issued by the CSIR in terms of Clause 3.2 above, which is governed by the contents of Clause 3; and
- v. Information which the Recipient is obliged to disclose in terms of a court order, subpoena or other legal process.

7.4 In the event that the Recipient is required by legal process to disclose any of the Confidential Information, covered by this clause 7, it shall provide the disclosing party with prompt notice of such requirements so as to enable the disclosing party to seek a protective order or waive compliance with the provisions of this clause. In the event that a protective order or other remedy is obtained, the Recipient shall use all reasonable efforts to ensure that only the information covered by such order or other remedy is disclosed. Whether or not a protective order or other remedy is obtained or a party has waived compliance with the provisions of this Contract, the Recipient shall take all reasonable steps to ensure that only that portion of the information that it is legally required to disclose is so disclosed.

#### **8. NO WARRANTIES**

CSIR does not warrant the merchantability or commercial viability of the work completed or deliverables as specified in the Proposal.

#### **9. LIMITATION OF LIABILITY**

Subject to the provisions of Clause 2.2 above, any claim for damages, including, but not limited to, loss of income, consequential or incidental damages, against the CSIR, whether in delict or based on this Contract, shall be limited to an amount equal to the contract price or the amount actually paid by the client to the CSIR in respect of the work performed in terms of this contract, whichever is the lesser.

#### **10. FORCE MAJEURE**

10.1 The CSIR shall not be responsible for any loss, injury, delay or damage or casualty suffered or incurred by the Client, because of the failure of the CSIR to comply or delay in complying with the terms of this Contract which are the result of causes beyond its reasonable control, including but not limited to natural calamities, strikes, fires, acts of government bodies, delays in use or sources of supply or any commercial impracticability of any nature whatsoever.

10.2 During any period of non-performance in terms of Clause 10.1, the relevant terms and conditions of this Contract will be suspended.

10.3 Should the duration of non-performance in terms of Clause 10.1, go beyond a period of 6 (SIX) months, either party may cancel the Contract, without any right of recourse as against each other, save in respect of work already executed.

#### **11. NON-WAIVER**

No relaxation or indulgence granted by the CSIR and no omission by the CSIR timeously or diligently to enforce any right under this agreement shall be deemed to amount to a waiver of that or any other rights.

#### **12. GOVERNING LAW AND DISPUTE RESOLUTION**

12.1 Regardless of the place of execution, performance or domicile of the parties, this Contract and all modifications and amendments thereof shall be governed by and construed under and in accordance with the laws of the Republic South Africa.

12.2 In the event of any dispute arising from this agreement, the dispute shall be adjudicated by a competent High Court in South Africa, (unless otherwise agreed between the parties at the time by means of a written arbitration or other agreement); and for these purposes the Parties agree to the exclusive jurisdiction of South African Courts for the adjudication of such disputes.

#### **13. BREACH AND TERMINATION**

13.1 In the event of any of the parties committing a material breach of any of the terms and conditions of this Contract, and remaining in default for a period of SEVEN (7) days after receipt by it of written notice from the other party calling for such breach to be remedied, the party delivering such notice shall be entitled, without prejudice to any other rights it may have in terms of this Contract or in law, to terminate this Contract by written notice to that effect given to the other party.

13.2 Any party may terminate this Contract at any time by giving to the other ("the defaulting party") notice of such termination if:

- i. the defaulting party is, other than for the purposes of reconstruction or amalgamation, placed under voluntary or compulsory liquidation or under judicial management or under receivership or under the equivalent of any of the foregoing;

- ii. a final and unappealable judgement against the defaulting party remains unsatisfied for a period of fourteen (14) days or more after it comes to the notice of the management of the defaulting party;
  - iii. the defaulting party make any arrangement or compromise with its creditors generally, or ceases, or threatens to cease, to carry on business;
- 13.3 The Contract may at any time be terminated by mutual and written consent between the parties.
- 13.4 Any termination of this Contract shall not absolve the parties from the obligation to observe the confidentiality measures and other restraints as set out herein. It is specifically recorded that the provisions of clauses 6, 7, 8 and 9 shall survive, in perpetuity, the termination of this Contract for whatever reason.
- 14. DOMICILIUM CITANDI ET EXECUTANDI**

The Parties hereto respectively choose as their domicilium citandi et executandi for all purposes of, and in connection with this Contract, the addresses stated in the attached Proposal accepted by the CSIR.
- 15. NOTICES**

Any notice to be given hereunder shall be given in writing and may be given either personally or may be sent by post, telex or facsimile and addressed to the relevant party at its domicilium or to such other address as shall be notified in writing by any of the parties to the other from time to time. Any notice given by post shall be deemed to have been served on the expiry of 7 (SEVEN) working days after same is posted by recorded delivery post or air mail. Any notice delivered personally or sent by telex or facsimile shall be deemed to have been served at the time of delivery or sending.
- 16. SEVERABILITY**

In the event that any one or more of the provisions of this Contract shall, for any reason, be held to be invalid, illegal or unenforceable in any respect, such invalidity, illegality or unenforceability shall not affect any other provisions of this Contract, but this Contract shall be construed as if such invalid, illegal or unenforceable provisions had never been contained herein, unless the deletion of such provision or provisions would result in the entire Contract being invalid, illegal or unenforceable.
- 17. VAT AND WITHOLDING TAX**
  - 17.1 Unless stated otherwise in the Proposal or agreed to by the parties, all fees and amounts stated in the Proposal are exclusive of Value Added Tax, which shall be paid by the Client upon submission of the relevant tax invoice.
  - 17.2 Any withholding tax as levied by any foreign country shall, where applicable, be for the account of and payable by the Client.
- 18. ELECTRONIC COMMUNICATIONS AND TRANSACTIONS ACT**
  - 18.1 No data message (as defined in the Electronic Communications and Transactions Act, 25 of 2002), including an e-mail, SMS, and recorded voice message, sent by the Client to CSIR, shall amend these contract conditions, or the rights and duties of the parties in any manner, unless such data message is reduced to paper and signed by both parties or their duly authorized signatories.
  - 18.2 Data messages (as defined above) sent by the Client to CSIR shall be deemed to be received by CSIR only when CSIR responds thereto, and for purposes of this clause an auto-response shall not be a response by CSIR.
  - 18.3 Legal notices and/or disclaimers linked to, accessible from or attached to a data message (as defined above) sent by CSIR to the Client shall be deemed part of these contract conditions and shall override and replace any such notices or disclaimers linked to, accessible from or attached to any data message sent by the Client in a return message.
- 19. ENTIRE AGREEMENT**
  - 19.1 This document, together with the Proposal, contains the entire contract between the parties and no party shall be bound by any undertaking, representation or warranty not recorded herein..
- 19.2 No alteration, variation, addition or agreed cancellation of this contract shall be of any force or effect unless reduced in writing and signed by both parties and their duly authorized signatorie

