The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952376

Communication Report

Deliverable D6.5

Project number - 952376 – VirA

Project funded by the European Union		
Dissemination Level		
PU	Public	X
PP	Restricted to other program participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Table of Contents

1. Summary

Communication report (due date – month 36 of the project) demonstrates results as well as effectiveness of communication activities, by evaluating awareness of the VirA project "Reducing networking gaps between Rīga Stradiņš University (RSU) and internationally leading counterparts in viral infection-induced autoimmunity research (VirA)" that is supported by the European Commission Horizon 2020 research and innovation funding programme. The report aims to elucidate comprehension of the key messages and whether stakeholders understood the call to action.

Project partners from Rīga Stradiņš University, University of Ferrara (Italy), Ulm University (Germany) and Zabludowicz Center for Autoimmune Diseases at the Sheba Medical Center have provided input in the project promotional activities described in the Communication Report.

2. Communication activities

Communication activities were conducted in accordance to the VirA project plan and communication strategy, that aimed to increase recognition and RSU brand in the international research community among prospective students and researchers, actively participate and share information in conferences and meetings, as well as inform stakeholders, such as institutions, the public, research communities, industry and policymakers through conferences, networking, workshops and other events. Stakeholders were invited to participate in workshops, seminars and conferences.

2.1. Webpage

All information about the project activities is available on the RSU website in English (Figure 1) https://www.rsu.lv/en/project/reducing-networking-gaps-between-riga-stradins-university-rsu-and-internationally-leading and

in Latvian https://www.rsu.lv/projekts/tiklosanas-nepilnibu-samazinasana-starp-rigas-stradina-universitati-rsu-un-starptautiski as well as in project web site https://vira-twinning.eu/ (Figure 2). Main events related to the VirA project are linked to the RSU webpage.

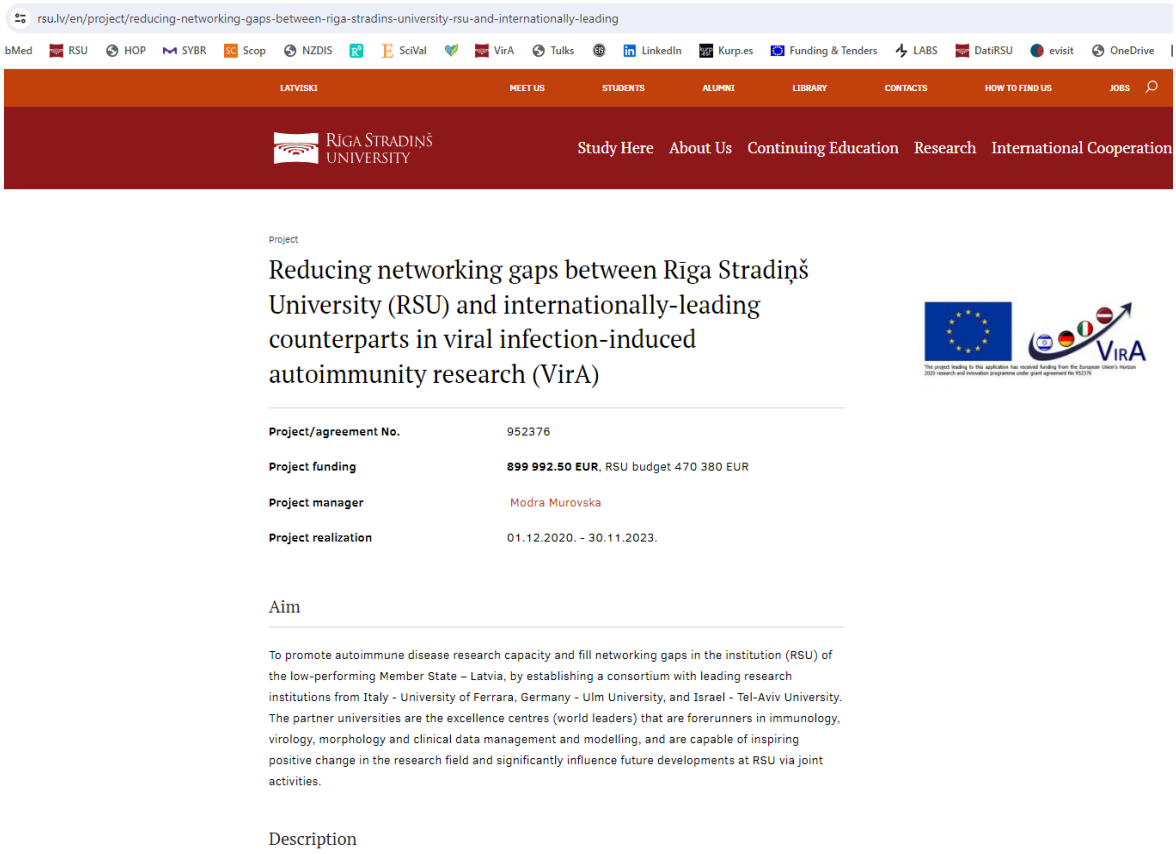


Figure 1. VirA project section on the RSU website web page www.rsu.lv.

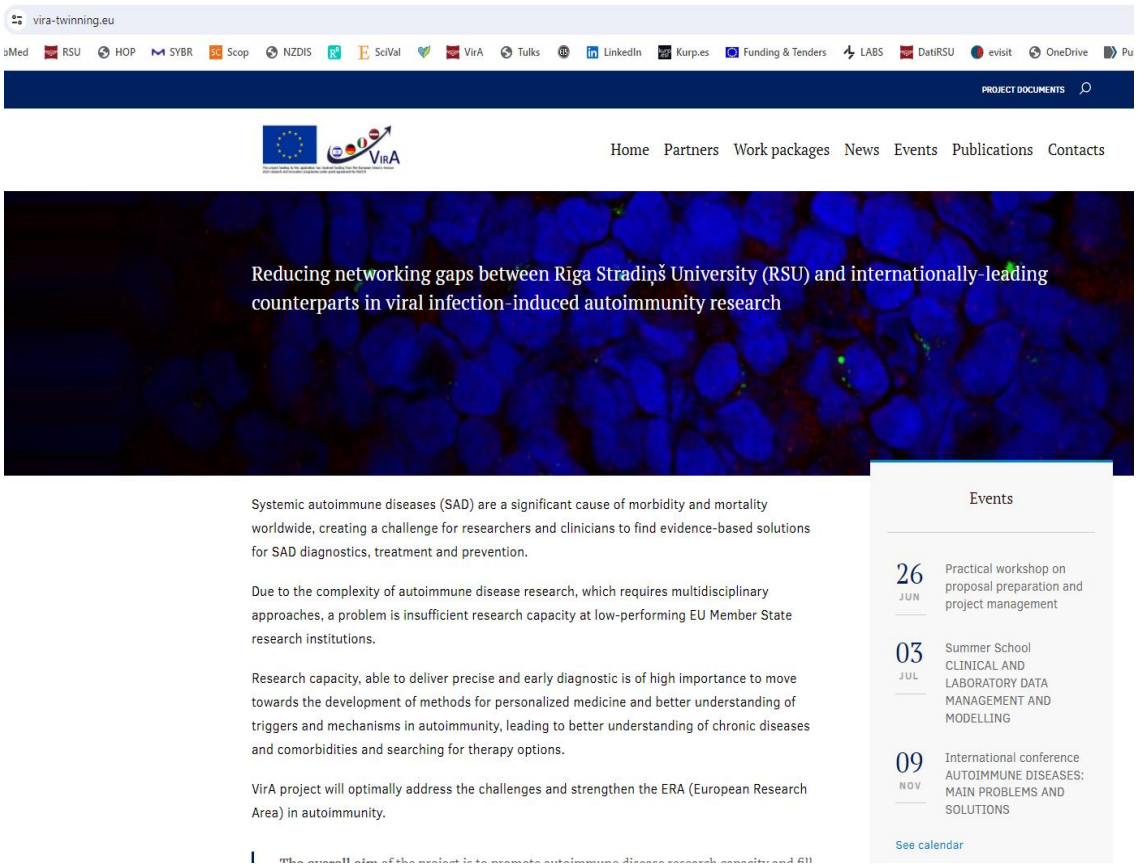


Figure 2. VirA project web page www.vira-twinning.eu section “Home”.

The webpage reflects the progress of the project, on-going activities and opportunities identified in the project. The webpage contains information on VirA project:

- Partners https://vira-twinning.eu/partners
- Work packages https://vira-twinning.eu/work-packages
- Project related news https://vira-twinning.eu/news
- Events https://vira-twinning.eu/events and past events https://vira-twinning.eu/events_archive
- Publications https://vira-twinning.eu/publications
- Contacts https://vira-twinning.eu/contacts

Each work package section contains also relevant reports of the project and links to public reports.

During the VirA project period from December 1, 2020 to November 27, 2023 the project sections at RSU website reached audience was 2125 pageviews (https://www.rsu.lv/en/project/reducing-networking-gaps-between-riga-stradins-university-rsu-and-internationally-leading):

Whereas VirA project website (https://vira-twinning.eu/) from December 1, 2020 to November 27, 2023 had 19 710 pageviews.

2.2. Flyer and brochure

Task 6.2 Preparing, publication and dissemination of the flyer and brochure on RSU autoimmune disease research [M1- M19] was led by RSU, involving all partners.

A special flyer was prepared to reflect the most important issues on RSU and VirA in an information-dense, but limited size format https://vira-twinning.eu/sites/default/files/imce/IPD-2653%20VirA%20buklets%20A4%20PDF.pdf . This was expanded in a special RSU brochure containing all information relevant to autoimmune disease research in RSU, areas of expertise and the potential direction of cooperation https://vira-twinning.eu/sites/default/files/imce/IPD-2653%20VirA%20bro%C5%A1%C5%ABra%20PDF.pdf . This brochure includes a special chapter on the VirA project.

Total number of printed copies was 300 brochures and 300 flyers. A digital version of the material instead of paper-based was considered useful for the dissemination activities, due to consequences of COVID-19 pandemics and opportunity to disseminate the information to broader stakeholder groups. The digital versions were also disseminated to all partners via e-mail, enabling further dissemination within their stakeholder groups.

The brochure containing information was updated in the middle of the project. The flyer and brochure are available at the project webpage https://vira-twinning.eu/work-packages/wp6-dissemination-external-communication-spreading-excellence

Due to the fact that both the VirA flyer and the VirA brochure are prepared in English and also in printed form, they are distributed at all international congresses and conferences attended by the implementers of the VirA project. In this way the information is distributed not only in VirA partner countries, but also, for example, in Bulgaria, Poland, Greece etc.:

- EATRIS-Plus Annual Meeting 2022 in Spain, Malaga. 9.05.2022. – 10.05.2022. Outgoing visit to University of Ferrara, Italy during 8.05.2022. – 27.05.2022.

- 2nd ISRAEL–LATVIA–ITALY Symposium of Autoimmunity, Nazareth, Israel, 20.05.2022. – 22.05.2022.

- 8th National Conference with International Participation “Morphological Days” Sofia, Bulgaria on 10.06.2022. – 12.06.2022.

- 13th International Congress on Autoimmunity in Athens, Greece, 10.06.2022. – 13.06.2022.

- 7th International Congress on Controversies in Rheumatology and Autoimmunity (CORA 2023) Turin, Italy,16.03.2023. -18.03.2023.

- Workshop 'Basic aspects of biostatistics, clinical and laboratory data management' Riga, Latvia, 23.02.2023. -24.02.2023.

- RSU Research Week 2023, Conference "Knowledge for Use in Practice" Riga, Latvia, 27.03.2023. – 31.03.2023.

- EATRIS Plus patient engagement Latvia node event in Riga, Latvia on 06.06.2023.

- 2nd Conference of the World Sociey for Virology (WSV): One Health - One World-One Virology. 15.06.2023. – 17.06.2023.

- International conference Autoimmune diseases: main problems and solutions, 9.11.2023. – 10.11.2023., Riga, Latvia.

- EATRIS 10-Year Anniversary Conference, The Hague, the Netherlands, 21.11.2023. – 22.11.2023.

In addition to the project-specific web-site, flyer and presentations in international conferences, also VirA project brochure and VirA posters are prepared and disseminated internationally (printed versions) in Spain, Italy, Israel, Bulgaria, Greece and the Netherlands with ongoing dissemination. In order to increase the global recognition, more tools are being used to reach international audiences.

2.3. Information to the research community

Task 6.4. was dedicated to providing information to the research community on topical issues in the field of autoimmune diseases, finding of new contacts and coordination of grants for conference attendance [M1-M36]. Task was led by RSU, involving all partners.

The visibility of the project with the purpose of increasing the global recognition of RSU was attained by preparing a special RSU - VirA poster (Figure 3) and banner (Figure 4) for presentation at major autoimmune disease events.

Figure 3. VirA project poster.

Figure 4. VirA project banner.

Following **communication channels** were used for providing information to the research community:

- Information of VirA project and events published in VirA web page https://vira-twinning.eu/

- Information of VirA project and events published in RSU web page https://www.rsu.lv/en/project/reducing-networking-gaps-between-riga-stradins-

university-rsu-and-internationally-leading (https://www.rsu.lv/projekts/tiklosanas-nepilnibu-samazinasana-starp-rigas-stradina-universitati-rsu-un-starptautiski) and in RSU event calendar https://www.rsu.lv/en/events;

• Information of VirA organised workshop and conference published in RSU Research Week Facebook profile
https://www.facebook.com/101348328415322/posts/369283124955173/?sfnsn=scwspmo

https://m.facebook.com/101348328415322/posts/450434023506749/?sfnsn=scwspmo

https://m.facebook.com/101348328415322/posts/502146545002163/

https://m.facebook.com/100031453283000/posts/pfbid02BVDVbVyRCj9WdgskssvZkx7iNRKDfqS9QC5YKWzQy59h4VXkvjaUsdRAvV2hhAprl/

https://m.facebook.com/story.php?story_fbid=pfbid0hTNswLJ6qFxeHnwuPER73CJ4Lw3JRnetjyQKxYbDkK1WgmtSm1EwEZJ8po8KFDCGl&id=100031453283000&_rdr

https://m.facebook.com/story.php?story_fbid=pfbid0hZhtctLHHSyRs6HVdAaJuUjY3zzbTPSazJLSyx8AzMv6HuKeQiusnd3JGAUiRRhxl&id=100031453283000;

• Information of VirA organised workshops and conference published in RSU LinkedIn profile (Figure 5)
https://www.linkedin.com/posts/riga-stradins-university_workshop-on-the-importance-of-differential-activity-6846060602264236032-RAEk/

https://www.linkedin.com/posts/riga-stradins-university_workshop-on-the-viral-infections-as-aetiological-activity-6863379797104500736-JjH7/

https://www.linkedin.com/posts/riga-stradins-university_within-the-framework-of-the-eu-research-and-activity-6940667227541012480-BCGF/?utm_source=linkedin_share&utm_medium=member_desktop_web

https://www.linkedin.com/feed/update/urn:li:activity:6981608400560222208/

https://www.linkedin.com/posts/riga-stradins-university_workshop-on-basic-aspects-of-biostatistics-activity-7029722979257245696-qGts/?utm_source=share&utm_medium=member_android

https://www.linkedin.com/posts/riga-stradins-university_eufunds-horizon2020-project-activity-7100471980893626368-cfan/?utm_source=share&utm_medium=member_desktop;

• Information of VirA organised workshops and conference is placed on RSU screens in university buildings (Figure 6);

• Information of VirA project and events is disseminated via e-mail;

• Information on the project disseminated via Rīga Stradiņš University newsletters – "*RSU Ziņas"* (RSU news), "*Universitātes Pulss*" (University pulse) (Figure 7 – 8) and "*Aktualitātes Pētniecībā*" (Topicalities in research);

• Information on VirA conference disseminated in EATRIS webpage https://eatris.eu/events/international-conference-autoimmune-diseases-main-problems-and-solutions/.

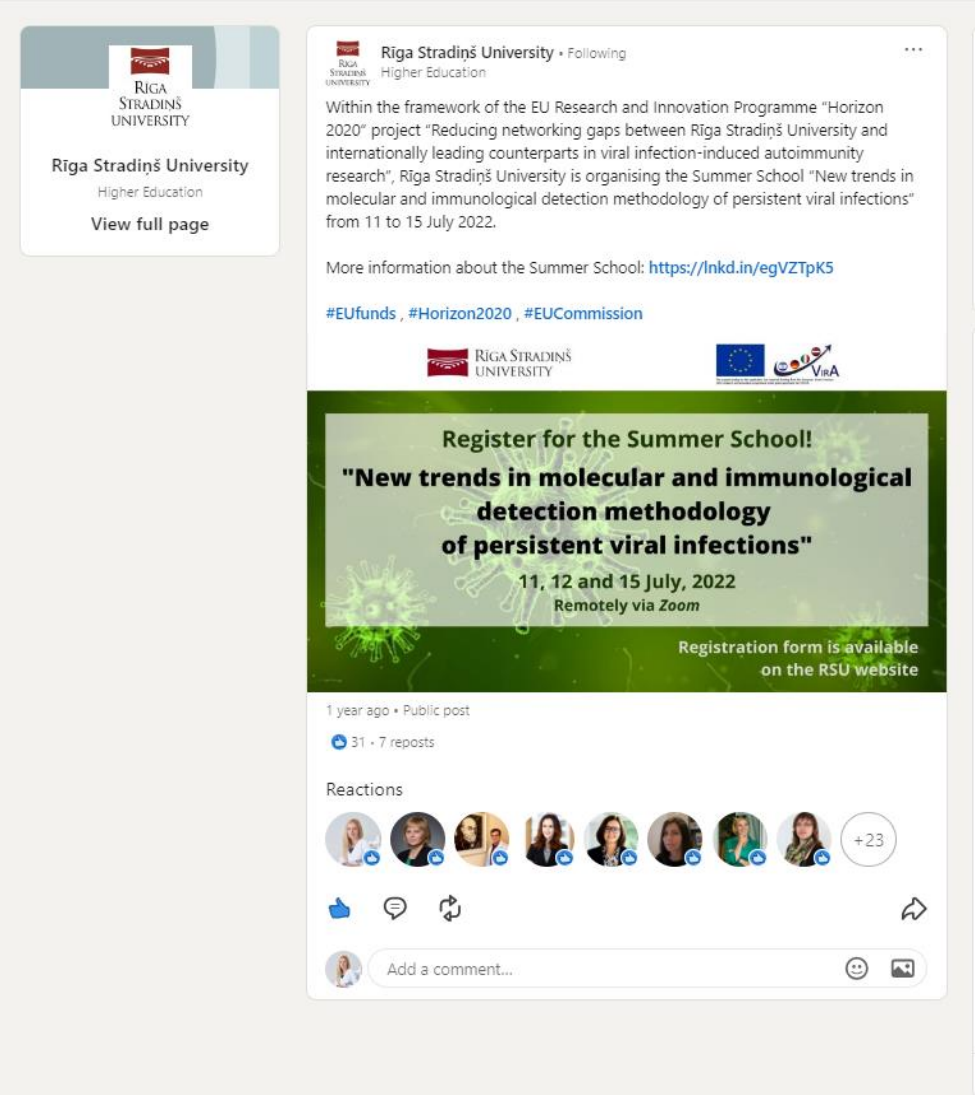


Figure 5. Example of VirA project information dissemination via LinkedIn social network

Figure 6. Example of information dissemination via RSU screens for VirA project related information

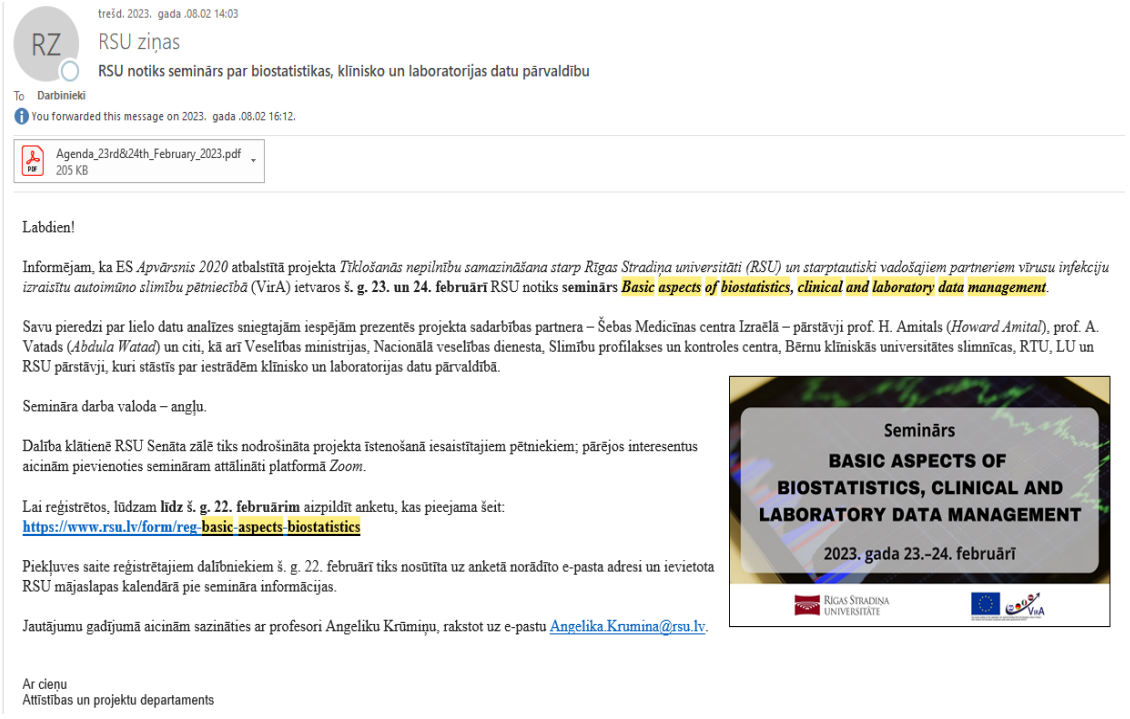


Figure 7. Example of information dissemination via Rīga Stradiņš University newsletter "RSU *Ziņas*"

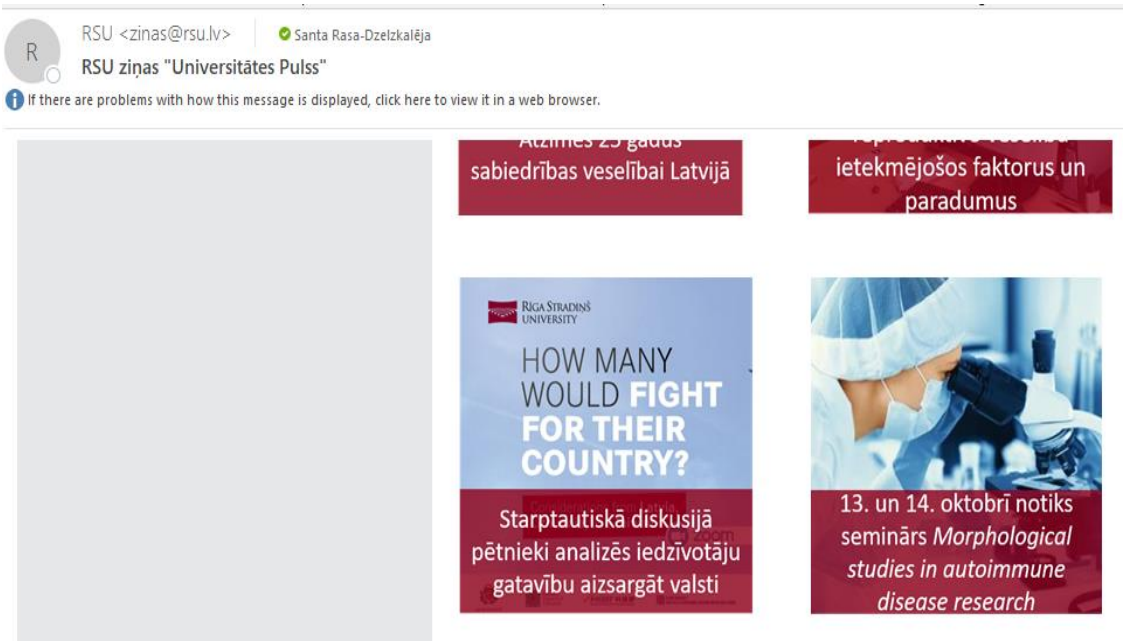


Figure 8. Example of information dissemination via Rīga Stradiņš University newsletter "*Universitātes Pulss*"

Sixteen grants for conference attendance grants, each 2000 EUR, were available within the VirA project for participation in international conferences aiming to increase the visibility of the project and the global recognition of RSU via presentations at major autoimmune disease events. Using this grant, participants attended several international conferences:

- 2nd ISRAEL – LATVIA – ITALY Symposium of Autoimmunity, Nazareth, Israel, 20.05.2022. – 22.05.2022. was attended by Modra Murovska, Zaiga Nora-Krūkle, Mihails Tarasovs, Šimons Svirskis, Anda Kadiša and Maksims Čistjakovs;

- 8th National Conference with International Participation "Morphological Days" Sofia, Bulgaria. 10.06.2022. – 12.06.2022 attended Sofija Semenistaja, Modra Murovska, Anda Kadiša;

- 13th International Congress on Autoimmunity in Athens, Greece on 10 – 13 June, 2022 was attended by Lība Sokolovska;

- 2nd Conference of the World Society for Virology (WSV): One Health - One World-One Virology. 15.06.2023. – 17.06.2023. attended Santa Rasa-Dzelzkalēja, Angelika Krūmiņa, Anda Vilmane, Irina Holodņuka (Irina Kholodnyuk), Šimons Svirskis, Lība Sokolovska, Maksims Čistjakovs, Valērija Groma , Zaiga Nora-Krūkle, Modra Murovska, Alīna Sultanova, Sabine Grāvelsiņa.

Detailed report on the conference attendance is available within the project deliverable D6.3. "Report on the participation in international conferences".

By e-mail and in VirA web page provided information to RSU employees on various other events, such as:

- Abstract submission for the 13th International Congress on Autoimmunity, The Mosaic of Autoimmunity, MAI Award,
- Weekly invitation to the Friday Mosaic of Autoimmunity International Zoom Meeting,
- 2nd Conference of the World Sociey for Virology (WSV): One Health - One World-One Virology
- 7th International Congress on Controversies in Rheumatology & Autoimmunity in Turin, Italy
- "RSU will host international conference Autoimmune Diseases: Key Challenges and Solutions" https://www.rsu.lv/en/news/rsu-will-host-international-conference-autoimmune-diseases-key-challenges-and-solutions.

2.4. Public media communication

Task 6.5. was carried out to ensure regular collaboration with public media to ensure flow of information [M1-M36].

The VirA project was reflected in the public media to win the confidence of the general public in autoimmune disease research at RSU and to increase the impact of research carried out at RSU among experts from other areas and the general public.

VirA project **video** was created as an informative film about the VirA project and introduction to the participants, activities and goals of this project. Video is available in:

- youtube.com https://www.youtube.com/watch?v=alkIsGoycFQ
- project webpage https://vira-twinning.eu/news/film-about-vira-project
- RSU webpage https://www.rsu.lv/en/project/reducing-networking-gaps-between-riga-stradins-university-rsu-and-internationally-leading (https://www.rsu.lv/projekts/tiklosanas-nepilnibu-samazinasana-starp-rigas-stradina-universitati-rsu-un-starptautiski)
- Facebook https://m.facebook.com/story.php?story_fbid=pfbid09UTDi3mqU4W8m4M79JtCX53q79Fyhb11L5YjUHYooih5yuiHsipx9fG74q3LP4xWl&id=100031453283000&_rdr
- LinkedIn https://www.linkedin.com/posts/riga-stradins-university_project-vira-reducing-networking-gaps-between-activity-7065566465445232640-0hLO?utm_source=share&utm_medium=member_desktop

Regular information updates were distributed via **press releases**. Released 4 press releases about VirA project:

- "Reducing networking gaps between Rīga Stradiņš University (RSU) and internationally- leading counterparts in viral infection–induced autoimmunity research" (VirA) https://vira-twinning.eu/news/press-release-15122020;

- "Rīga Stradiņš University (RSU) is continuing to implement an international project on Autoimmune Diseases" https://vira-twinning.eu/news/press-release-20122021;

- "RSU Implements Ambitious International Project on Cooperation in Research on Autoimmune Diseases Caused by Viruses" https://vira-twinning.eu/news/press-release-22022023;

- "RSU will host international conference Autoimmune Diseases: Key Challenges and Solutions" https://www.rsu.lv/en/news/rsu-will-host-international-conference-autoimmune-diseases-key-challenges-and-solutions.

Professor Modra Murovska gave **interviews:**

- "COST's inclusivity initiatives drive excellence" 7/09/22; Cost.eu, Belgium, https://www.cost.eu/inclusivity-excellence/;
- Newspaper "Saldus Zeme" No. 4 (National), Latvia, 13/01/23 "At the moment - the festival of three viruses";
- Latvijas Radio 4 (National), Latvia, Radio Домская площадь Латвия и Китай: такой разный ковид?", 12/01/23; https://lr4.lsm.lv/lv/lr4/peredachi/domskaja-ploschad/;
- Latvijas Radio (Radio of Latvia), in addition also *LSM* (Latvian Public Media) article is published https://www.lsm.lv/raksts/dzive--stils/tehnologijas-un-zinatne/15.06.2023-pasaules-virusologi-konference-riga-spriez-par-gatavibu-nakamajai-pandemijai.a512948/;
- LR2 (National), Latvia, Radio, program "Nākotnes pietura (Future Stop)" 6/07/23 – "Is COVID just a little dormant or has it disappeared completely?" https://replay.lsm.lv/lv/ieraksts/lr/179020/modra-murovska-vai-covids-tikai-nedaudz-iesnaudies-vai-pazudis-pavisam;
- MEDICAL NEWS "Can Long COVID Lift the Veil on Chronic Fatigue Syndrome?" 29/09/23 Univadis from Medscape (International), Latvia, Web https://www.univadis.com/viewarticle/can-long-covid-lift-veil-chronic-fatigue-syndrome-2023a1000nuj

Professor Angelika Krūmiņa published article on journal "*Latvijas Ārsts*" (Latvian Doctor) reaching stakeholder group of clinicians https://science.rsu.lv/en/publications/aktualit%C4%81tes-mial%C4%A3isk%C4%81-encefalomiel%C4%ABta-hronisk%C4%81-noguruma-sindroma.

Regular **e-newsletters** were published within Rīga Stradiņš University newsletters – "*RSU Ziņas"* (RSU news), "*Universitātes Pulss*" (University pulse) and "*Aktualitātes Pētniecībā*" (Topicalities in research) that raised awareness about VirA's activities. Publication date: 24.09.2021., 22.10.2022., 05.11.2022., 10.06.2022., 29.06.2022., 30.09.2022., 17.02.2023., 30.06.2023., 01.09.2023., 07.11.2023.

Social media (RSU Research Week Facebook profile and RSU LinkedIn profile) were used to communicate VirA project activities adding such hashtags as: #EUfunds, #Horizon2020, #project, #EUCommission, #autoimmunediseases, #viralinfections, #RSU, #research

2.5. Popular-scientific communication

Task 6.6. aimed at providing research information for popular-scientific events in entertaining manner [M17-M36].

Presentations in popular-scientific events involving students and schoolchildren were organized in order to attract new research potential to autoimmune disease research. In addition, this enhanced public perception of RSU and reliance on the expertise of RSU.

Popular-scientific seminar with 3 presentations by MSc Lība Sokolovska, PhD Santa Rasa-Dzelzkalēja and PhD Šimons Svirskis for 11th grade schoolchildren of Jelgava State Gymnasium was held on 10.12.2021.

Within the Junior Achievement Career Education Program "*Ēnu diena*" (Shadow Day) Riga State Gymnasium No. 1, 10th grade schoolchildren attended the Institute of Microbiology and Virology to gain knowledge on research on 6.04.2022.

A special target group – patient organizations was reached in the EATRIS Plus patient engagement Latvia node event in Riga, Latvia on 06.06.2023.

VirA project video is available in youtube.com https://www.youtube.com/watch?v=alkIsGoycFQ , reaching broad range of stakeholders, including general public.

2.6. Events

VirA project was communicated within various events:

- Presentation on VirA project at the "Research Breakfast" at Rīga Stradiņš University by Prof. Modra Murovska https://www.rsu.lv/notikumi/zinatnieku-brokastis-rsu-zinatnieku-vaditas-sadarbibas-un-infrastrukturas-platformas

- COST conference. Prof. Uldis Berķis presented “COST Action as a springboard to a successful Twinning proposal”. Presentation
- COST Annual Report 2021, pp 19 (https://www.cost.eu/uploads/2022/04/COST_Annual_report_2021.pdf)
- Rīga Stradiņš University (RSU) and Ariel University (Israel) sign a memorandum of understanding (MoU) on 29.03.2022. https://www.rsu.lv/en/news/rsu-signs-memorandum-understanding-ariel-university

- Research Breakfast. Discussion 'Setting up priorities in the field of autoimmune disease research in Riga Stradiņš University' moderated by Prof. Yehuda Shoenfeld, President of Ariel University, Head of Zabludowicz Center for Autoimmune Diseases, Sheba Medical Centre (Israel) and attended by Zaiga Nora-Krūkle, Modra Murovska and other VirA personnel (05.10.2022) https://www.rsu.lv/en/events/research-breakfast-discussion-setting-priorities-field-autoimmune-disease-research-riga

- RSU Strengthens Cooperation with Sheba Medical Centre in Israel on 29.11.2022. https://www.rsu.lv/en/news/rsu-strengthens-cooperation-sheba-medical-centre-israel

- VirA banner presentation at conference "*Apvārsnis Eiropa: Latvijas ceļš uz zinātnes izcilību*" (Horizon Europe: Latvia's path to scientific excellence) by Asja Lunga.
- 1st LATVIA - ISRAEL On-line Symposium of Autoimmunity, Zoom Platform, 16.03.2021
- RSU Research week 2021: Knowledge for Use in Practice, Riga, Latvia 24.03.2021. - 26.03.2021.
- 12th International Congress on Autoimmunity, 28.05.2021. – 1.06.2021.
- World Society for Virology first international conference: Tackling Global Viral Epidemics Conference 2021. 16.06.2021. - 18.06.2021.
- 15th Biennial Congress European Association of Oral Medicine 23.09.2021. - 25.09. 2021.
- XXVIII Congress of Polish Physiological Society, 15.09.2021. -17.09.2021.
- Autoimmunity, COVID and Post-COVID International Congress: Updates on Autoimmune Clinical Conditions, COVID-19 and Post-COVID Syndrome, 27.11.2021. - 28.11.2021.
- ISPOR (Professional Society for Health Economics and Outcomes Research) Conference 2022: The Future of HEOR in Patient-Driven Digital Healthcare Systems, United States, Washington, 16.05.2022. – 18.05.2022.
- 2nd ISRAEL – LATVIA – ITALY Symposium of Autoimmunity, Nazareth, Israel, 20.05.2022. – 22.05.2022.
- 8th National Conference with International Participation “Morphological Days” Sofia, Bulgaria. 10.06.2022. -12.06.2022.
- 13th International Congress on Autoimmunity in Athens, Greece, 10.06.2022. – 13.06.2022.
- 8th Annual International Conference on Public Health, Athens, Greece, 20.06.2022.
- 15th Medical and Scientific Conference of the International Association for Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis (IACFS/ME), United States, 27.07.2022. – 30.07.2022.
- 4th POLYTHEMATIC Panhellenic Congress of Autoimmune Diseases, Rheumatology and Clinical Immunology. 9.09.2022 - 11.09.2022.
- International Consortium for Health Outcomes Measurement (ICHOM) Conference: Outcomes Driving Positive Change: The New Era of Healthcare, Boston, United States, 02.11.2022.
- ISPOR (Professional Society for Health Economics and Outcomes Research) Europe Conference 2022: Collaborating Across Borders: Building & Using Evidence to Enable Access. Vienna, Austria, 8.11.2022.
- 7th International Congress on Controversies in Rheumatology and Autoimmunity (CORA 2023) Turin, Italy, March 16.03.2023. -18.03.2023.
- RSU Research Week 2023, Riga, Latvia, 27.03.2023. – 31.03.2023.
- 2nd Conference of the World Society for Virology (WSV): One Health - One World-One Virology. 15.06.2023. – 17.06.2023.
- *Pasaules latviešu zinātnieku V kongress "Zinātne Latvijai"* (5th World Congress of Latvian Scientists "Science for Latvia"), Riga, Latvia, 29.06.2023.
- Friday Mosaic of Autoimmunity International e-Meetings (weekly on Fridays). Online via Zoom platform.

- International conference “Autoimmune diseases: main problems and solutions”, 9.11.2023. – 10.11.2023., Riga, Latvia.

2.7. CORDIS

VirA project information and reports are published at Community Research and Development Information Service (CORDIS) - European Commission's primary public repository and portal to disseminate information on all European Union (EU) funded research projects and their results in the broadest sense. https://cordis.europa.eu/project/id/952376

2.8. Practical course

Since the beginning of VirA, Germany team (Prof. M. Schneider / Dr. C. Scheiber / H. Bauer) perform a yearly practical course for 25-30 students of Molecular Medicine within 2 weeks in February. The design of this course is to explain why hyperinflammatory conditions and genetic polymorphisms (TNF-a and IL-6) may lead to autoimmunity and immune dysfunction. Students need to pass an oral examination at the end of the semester. The course is held in German with 4 talks on theoretical and practical aspects of individual patients tested by whole blood stimulation assays. The research aspects and the practical aspects are explained in the protocols attached (Annex 1).

3. Research articles publication grant

Research articles publication grants whose total amount was 8000 EUR was used to cover costs for publications authored by VirA project participants/partners:

Araja D, Krumina A, Nora-Krukle Z, Schneider ME, Berkis U, Murovska M. Coaching to strengthen critical success factors in integrative care for chronic fatigue patients: the Patient Needs-Resources Model. Front Neurosci. 2023 Jul 21;17:1202930. doi: 10.3389/fnins.2023.1202930. PMID: 37547141; PMCID: PMC10400772.
https://www.frontiersin.org/articles/10.3389/fnins.2023.1202930/full

Gravelsina S, Vilmane A, Svirskis S, Rasa-Dzelzkaleja S, Nora-Krukle Z, Vecvagare K, Krumina A, Leineman I, Shoenfeld Y, Murovska M. Biomarkers in the diagnostic algorithm of myalgic encephalomyelitis/chronic fatigue syndrome. Front Immunol. 2022 Oct 10;13:928945. doi: 10.3389/fimmu.2022.928945. PMID: 36300129; PMCID: PMC9589447.
https://www.frontiersin.org/articles/10.3389/fimmu.2022.928945/full

Rasa-Dzelzkaleja S, Krumina A, Capenko S, Nora-Krukle Z, Gravelsina S, Vilmane A, Ievina L, Shoenfeld Y, Murovska M; VirA project. The persistent viral infections in the development and severity of myalgic encephalomyelitis/chronic fatigue syndrome. J Transl Med. 2023 Jan 18;21(1):33. doi: 10.1186/s12967-023-03887-0. PMID: 36653846; PMCID: PMC9847171.
https://translational-medicine.biomedcentral.com/articles/10.1186/s12967-023-03887-0

Soffritti I, Gravelsina S, D'Accolti M, Bini F, Mazziga E, Vilmane A, Rasa-Dzelzkaleja S, Nora-Krukle Z, Krumina A, Murovska M, Caselli E. Circulating miRNAs Expression in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Int J Mol Sci. 2023 Jun 24;24(13):10582. doi: 10.3390/ijms241310582. PMID: 37445763; PMCID: PMC10341915.
https://www.mdpi.com/1422-0067/24/13/10582

Čēma I, Kakar J, Dzudzilo M, Murovska M. on behalf of VirA Project Nr 952376. Immunological Aspects of EBV and Oral Mucosa Interactions in Oral Lichen Planus. Appl. Sci. 2023, 13(11), 6735; https://doi.org/10.3390/app13116735

4. Conclusion

Communication of VirA project “Reducing networking gaps between Rīga Stradiņš University (RSU) and internationally leading counterparts in viral infection-induced autoimmunity research (VirA)” activities are very important, not only because they help to spread information about the project and increase positive recognition, but also facilitates finding of new potential partners.

This project produced scientific research publications, conference proceedings, presentations, material for social networks and media, studies, reports and other materials. These are disseminated according to the best practices in the research field, moreover most of the materials are open access.

The accomplished VirA project communication raised awareness about VirA project, its activities and results, increased knowledge in research, increased the global recognition of RSU, gained the confidence of the general public in autoimmune disease research at RSU and increased the impact of the research among experts from other areas.

Klinik für Anästhesiologie und Intensivmedizin
Prof. E. Barth, Prof. M. Weiß
Chirurgie CKL Labore C1.593 / C1.659, C1.662, C1.667
Prof. M. Schneider / Dr. C. Scheiber / H. Bauer

TNF-α / IL-6 release and functional SNPs (single nucleotide polymorphisms)

BACKGROUND[1,2]

TNF-α is one of the most important mediators of inflammation. The cell types secreting highest concentrations of TNF-α are monocytes or macrophages [1]. A strong inducer for TNF-α release / expression is bacterial endotoxin (LPS), stimulating a signaling cascade via TLR-4. The counterpart of TNF-α in lymphocytes lymphotoxin A (LTA, TNF-β), which is structurally and functionally similar to TNF-α and also guides lymph node formation [2].

Earliest effects by TNF-α have been described in the context of **tumor necrosis** [1]. Later, TNF-α has been shown to promote tumor growth and today, chronically elevated TNF-α may increase the manifestation or progression of malignancies and promote autoimmune disease manifestation.

TNF-α is also responsible for wasting syndromes also called **cachexia** [2]. The responsiveness of a patient to secrete TNF-α is a measure for the intensity of an inflammatory response. Part of the intensity of the immune response is related to a **single nucleotide polymorphism in the TNF-α promotor at position -308**. The SNP rs1800629 (-308 G/A) has been reported to influence the course of sepsis [3, 4, 5]. Furthermore, this SNP has been associated with an increased risk for Morbus Crohn [6] as well as pathologies in lipid metabolism [7].

IL-6 is another inflammatory cytokine. It is synthesized in a local lesion in the initial stage of inflammation, from where it reaches the liver via the bloodstream and induces a wide array of acute phase proteins like C-reactive protein **(CRP)**, serum amyloid A, fibrinogen and others [8]. The **IL-6 SNP rs1800795 (-174 C/G)** has been shown to be associated with differing levels of cytokine transcription and production. Studies showed that patients carrying the **C-allele exhibited lower IL-6 serum concentrations and a higher IL-6 secretion after LPS-stimulation [9].** It was further shown, that the IL-6-174 C/G polymorphism is – among several others - associated with pregnancy-associated complications leading to spontaneous abortion [10].

Studies revealed that the presence the promotor variants of TNF-α-G308A and IL-6-G174C are associated with an increased risk of sepsis developing into a septic shock [11], as well as organ failure and mortality related to influenza virus infections [12], and severe COVID-19 disease [13].

In our experiment we intend to find out, whether a promoter polymorphism of TNF-α affects the endotoxin response of patients with systemic inflammation (SIRS) or sepsis.

References

1. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 2003;3:745-756.
2. Croft M. The role of TNF superfamily members in T-cell function and diseases. Nat Rev Immunol. 2009;9:271-285.
3. Teuffel O, Ethier MC, Beyene J, Sung L. Association between tumor necrosis factor-alpha promoter -308 A/G polymorphism and susceptibility to sepsis and sepsis mortality: a systematic review and meta-analysis. Crit Care Med;38:276-282.
4. Zhang Y, Cui X, Ning L, Wei D. The effects of tumor necrosis factor-α (TNF-α) rs1800629 and rs361525 polymorphisms on sepsis risk. *Oncotarget*. 2017;8(67):111456–111469. Published 2017 Nov 30.
5. Acar L, Atalan N, Karagedik EH, Ergen A. Tumour Necrosis Factor-alpha and Nuclear Factor-kappa B Gene Variants in Sepsis. *Balkan Med J*. 2018;35(1):30–35. doi:10.4274/balkanmedj.2017.0246
6. Ferreira AC, Almeida S, Tavares M, Canedo P, Pereira F, Regalo G, Figueiredo C, Trindade E, Seruca R, Carneiro F, Amil J, Machado JC, Tavarela-Veloso F. NOD2/CARD15 and TNFA, but not IL1B and IL1RN, are associated with Crohn's disease. Inflamm Bowel Dis. 2005 Apr;11(4):331-9.
7. Leońska-Duniec A, Ficek K, Świtała K, Cięszczyk P. Association of the TNF-α -308G/A polymorphism with lipid profile changes in response to aerobic training program. Biol Sport. 2019 Sep;36(3):291-296. doi: 10.5114/biolsport.2019.85456. Epub 2019 Jul 31. PMID: 31624424; PMCID: PMC6786320.
8. Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harbor Perspectives in Biology*. 2014;6(10):a016295.
9. Jiménez-Sousa MA, Medrano LM, Liu P, et al. *IL-6* rs1800795 polymorphism is associated with septic shock-related death in patients who underwent major surgery: a preliminary retrospective study. *Annals of Intensive Care*. 2017;7:22.
10. Alkhuriji AF, Al Omar SY, Babay ZA, El-Khadragy MF, Mansour LA, Alharbi WG, Khalil MI. Association of IL-1β, IL-6, TNF-α, and TGFβ1 Gene Polymorphisms with Recurrent Spontaneous Abortion in Polycystic Ovary Syndrome. Dis Markers. 2020 May 5;2020:6076274. doi: 10.1155/2020/6076274. PMID: 32454906; PMCID: PMC7232732.
11. Feng B, Mao ZR, Pang K, Zhang SL, Li L. Association of tumor necrosis factor α -308G/A and interleukin-6 -174G/C gene polymorphism with pneumonia-induced sepsis. J Crit Care. 2015 Oct;30(5):920-3.
12. Elsayed SM, Hassanein OM, Hassan NHA. Influenza A (H1N1) virus infection and TNF-308, IL6, and IL8 polymorphisms in Egyptian population: a case-control study. J Basic Appl Zool. 2019;80(1):61. doi:10.1186/s41936-019-0131-1
13. Rokni, M., Sarhadi, M., Heidari Nia, M., Mohamed Khosroshahi, L., Asghari, S., Sargazi, S., Mirinejad, S., & Saravani, R. (2022). Single nucleotide polymorphisms located in TNFA, IL1RN, IL6R, and IL6 genes are associated with COVID-19 risk and severity in an Iranian population. Cell Biology International, 46, 1109– 1127. https://doi.org/10.1002/cbin.11807

Klinik für Anästhesiologie und Intensivmedizin
Prof. E. Barth, Prof. M. Weiß
Chirurgie CKL Labore C1.593 / C1.659, C1.662, C1.667
Prof. M. Schneider / Dr. C. Scheiber / H. Bauer

PART I

Ex Vivo Whole Blood TNF-α Secretion Assay - TruCulture®

(https://myriadrbm.com/truculture/)

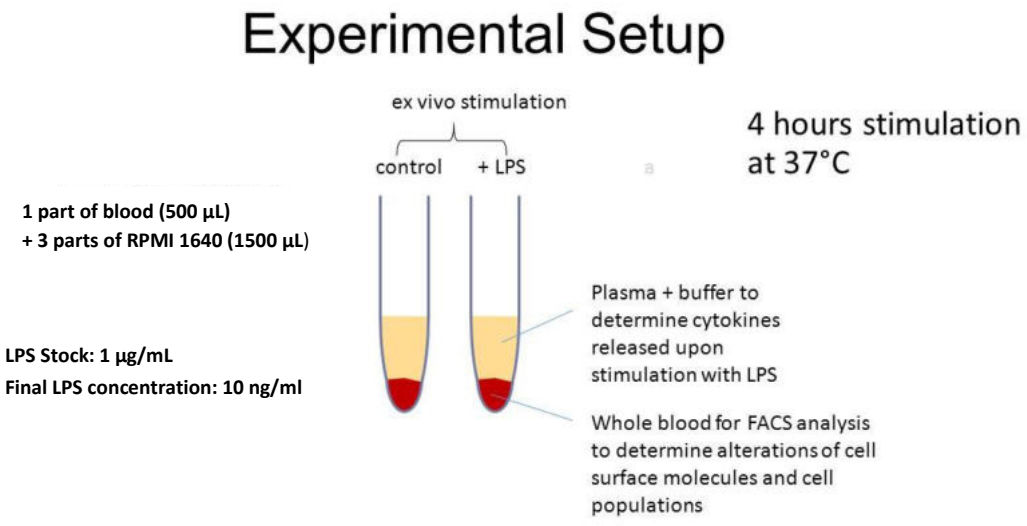
TruCulture® is a commercially available test to quantify the amount of secreted TNF α and IL-6 in a whole blood assay following stimulation with a standardized lipopolysaccharide (LPS).
The amount of TNF-α produced, can be used as a marker of fitness, hyperactivation or suppression of a patient's immune system. The amount of released cytokine is further dependent on a functional SNP mutation of the TNF-α gene.

Procedure: Heparinized whole blood is taken from patients treated on ICU (intensive care) almost every 2 h to test blood gases, pH, glucose, lactate, C-reactive protein and other markers. We may use the left-over blood samples for our practical Immunology Course.

Set-up of whole blood stimulation and take care of our hygiene concepts. Consider the correct desinfection solutions:
Video: 07 Reinigungsmittel

Before setting up the experiment, we need to anonymize the blood sample:
Video: 01 Registrierung der Blutprobe

Now, we do the whole blood stimulation assay:
Video: 02 Vollblutstimulation

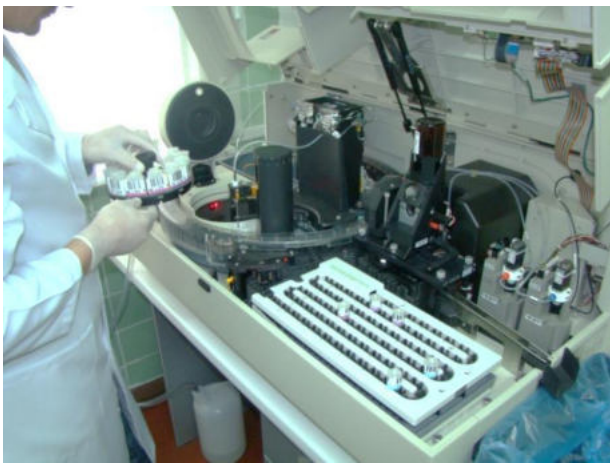


All videos: https://www.youtube.com/playlist?list=PLQgq-er8mIN_IDdw57wa7krvXwSBd_kew

After incubation at 37 °C for 4 h, the supernatant is collected and tested for the amount of TNF-α released, by a semi automated ELISA (Immulite®, siemens.com).
How to do this is shown in
Video: 03: Mischen und Zentrifugieren

And prepare the cell-free supernatant to determine their cytokine content:
Video: 04 Abnahme von Überstand und Eppendorf-

Immulite 1000®:
Is a chemiluminescent Immunoassay System. The sample is pipette into the incubation vial. An automated robot adds the sample into another vial containing a polystyrene bead with anti-TNF-α antibodies. After washing, a secondary ALP-labelled antibody is added. Following incubation, a substrate is added and chemiluminescent photons are generated correlating with the TNF-α concentration of the original sample.



http://neolab.kiev.ua/gallery/images/immulite.jpg

https://rbm.myriad.com/files/2013/04/TruCulture-header.jpg

Video:

Video: 05 Proben in Immulite

Similar to our experimental set up, clinical studies use a more standardized system to test the immune competence of a patient. This ex vivo whole blood stimulation is named *TruCulture®* and has been invented by HOTScreen GmbH (formerly EDI GmbH) in Germany. The system is distributed by Myriad RBM in the U.S. It can be also used to test the effect of chemotherapeutics, toxins and nutritional substances on the immune system.
You can document your cytokine results in this table and also add the genotype results obtained in part II:

The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952376

Read Results of the Immulite 1000® assay:

KeyPat Id	No stimulus [pg/ml]	Stimulation with LPS [pg/ml]

Results of SNP genotyping: Group Name:

Patient [name or number]	TNF-α genotype (-308)	[TNF-α] (Truculture)	IL-6 genotype (-174)	[IL-6] (Truculture)

PART II

Isolation of genomic DNA from whole blood (Maxwell 16®)

DNA will be isolated from 300 µl of heparinized blood of the respective patient (pseudonymized by the KeyPat-Id number). The Maxwell 16® (*#AS1290,* Promega®) is an automated purification system, which isolates different nucleic acids from a variety of samples. We will use the Maxwell 16® LEV Blood DNA Kit to isolate genomic DNA from our blood samples. First, the blood is mixed for approximately five minutes. Then, 300 µl Lysis Buffer and 30 µl Proteinase K, which are supplied in the Kit, are added to 300 µl of the blood sample. The samples are vortexed for about 10 seconds and incubated at 56°C for 20 min. The high temperature and the detergents Guanidinium thiocyanate (50-75 %) and Polyethylene glycol tert-octyl-phenyl ether (Triton-X, < 2 %), which are components of the lysis buffer, lead to lysis of cell and nuclear membranes. The Proteinase K degrades various proteins. Histones are also degraded, enabling the release of the genomic DNA. After the incubation time, the whole batch is pipetted into well No.1 of the cartridge. The cartridges are placed into the cartridge holder with a plunger and an additional tube, containing 60 µl elution buffer. The cartridge holder is then placed inside the Maxwell 16® Instrument and the machine is started. The Maxwell 16® instrument uses paramagnetic particles, the MagnaCel™ particle, which takes advantage of the silica binding capacity of nucleic acids. During the automated purification process. Genomic DNA is bound to the paramagnetic particle, washed with ethanol and eventually released into the elution buffer.

Video: Maxwell16

DNA Quantification (Nanodrop®)

For quantification of the DNA yield from the 300µl blood, the extinction at a wavelength of 260/280 nm is determined using a miniaturized spectrophotometer (Nanodrop® nd-1000, Thermo Scientific). The purine and pyrimidine bases of nucleic acids absorb 260 nm light strongly.

The specific extinction coefficient of a given DNA solution depends on various variables, such as its nucleotide sequence or the pH of the solution as well as contaminating proteins.

In average the extinction coefficient of DNA is ε = 0.02 $(\mu g/ml)^{-1} * cm^{-1}$. The concentration of a DNA solution can be calculated quite accurately with this value and the measured absorption value, using the following formula.

$$c\ (ng/\mu l) = A_{260\ nm} / (\varepsilon_{260} * d)$$

A = Absorption in AU at
e = extinction coefficient in $(\mu g/ml)^{-1} * cm^{-1}$ at 260 nm
b = path length in cm

The absorption maximum of aromatic side chains of amino acids is at 280 nm. In order to assess the purity of the nucleic acid solution, the ratio of absorption at 260 nm to 280 nm can be calculated. DNA is considered pure, if the ratio is approximately 1.8. In the presence of protein contamination, the ratio will strongly decrease.

Quantitative polymerase chain reaction (qPCR) for SNP analysis using predesigned TaqMan® SNP Genotyping Assays

The aim of a PCR is to determine smallest amounts of DNA by repeated amplification (standard protocols basically use n=40 cycles) of the target sequence of interest using a reaction mix containing dNTPs, sequence-specific primers, reaction buffer and a heat-stable DNA polymerase.

A qPCR (*quantitative* PCR) differs in that way, in which a complementary target sequence (*probe*) is added to the master mix. The probe itself is labelled with a fluorescent dye, whose signal is constantly detected during amplification. The software connected with the qPCR cycler is able to generate amplification curves in real time, based on the increase of the fluorescence signal. Derived from the amplification curves, the software determines for each sample a so-called CT (*cycle threshold*) value. The CT value indicates the cycle, in which the increase in fluorescence becomes exponential. Hence, a lower CT value indicates a higher amount of the specific target.

This procedure of **qPCR analysis can further be used to determine single nucleotide polymorphisms (SNPs)** in a given DNA sample.

In our practical course, we use predesigned SNP assays for analyzing the previously mentioned TNA-α and IL-6 promotor SNPs in the gDNA extracted from the patients' blood samples.

The principle of SNP genotyping using TaqMan® Assays is shown in Figure 1. In this case, the reaction mix contains *two* probes (one for each allele), labelled with different fluorescent dyes (FAM® / Fluorescein amitide, and VIC® / victoria) on their 5' end. The reaction mix is completed by forward- and reverse- primers, a heat-stable DNA polymerase (AmpliTaq Gold® DNA Polymerase), and further mixed with extracted gDNA of interest. After annealing of the primers, the AmpliTag Gold® DNA Polymerase starts amplification. By reaching the probe (annealed to the specific SNP region) the exonuclease activity of the Polymerase cleaves the reporter dye from the probe which results in an allele-specific increase of fluorescent signal. To prevent the reporter dyes to emit fluorescence signals before cleaving, each probe contains a nonfluorescent quencher molecule at their 3' end, preventing unspecific background fluorescence. The quencher is further bound to a Minor Groove Binder (MGB) molecule. The latter binds the minor groove of the DNA, resulting in increased stability of the amplification complex, and also allows the use of short-length probe molecules (13 bp).

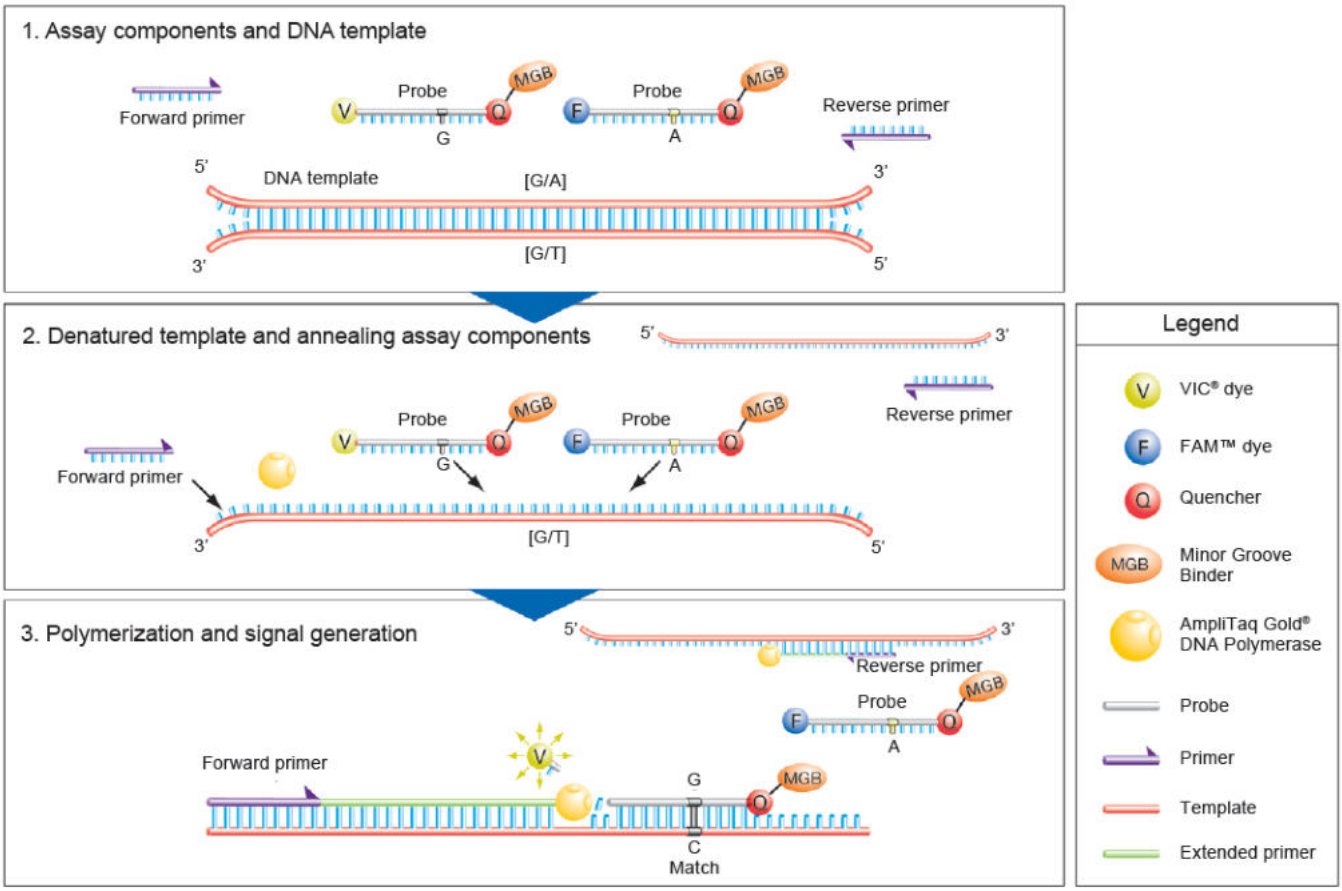


Figure 1. Allelic discrimination is achieved by the selective annealing of TaqMan® MGB probes.

The handling procedure is shown in this

Video: SNP detection by qPCR

After the qPCR run is finished, the software generates a scatter plot for all analyzed samples. Exemplarily, Figure 2 shows the scatter plot for the IL-6 SNP from one group of last year's practical course. The relative fluorescence signals of both dyes (x axis: C allele; y-axis: G allele) are plotted against each other for each sample, which typically results in three possible cluster patterns.

Samples showing high fluorescence signals for only one allele are plotted either in the lower right or upper left corner (depending on the allele-specific dye) and are homozygous for the respective allele (y-axis: blue dots, homozygous G/G, wildtype; x-axis: red dots, homozygously mutated C/C). Accordingly, samples with the heterozygous genotype usually show fluorescent signals for both alleles, however each to a minor intensity.

This procedure results in a scatter plot in between the clusters for the homozygous alleles, usually in a 45°-like angle from the lower left corner (green dots, heterozygous). To be sure the fluorescent signal(s) are not generated due to background fluorescence, every run contains a so-called ***No-template control*** **(NTC),** in which the gDNA template is replaced by nuclease-free water. These NTCs have to show no fluorescence emission signal(s) for any dye, and should usually be located in the lower left corner (black dots, undetermined signal).

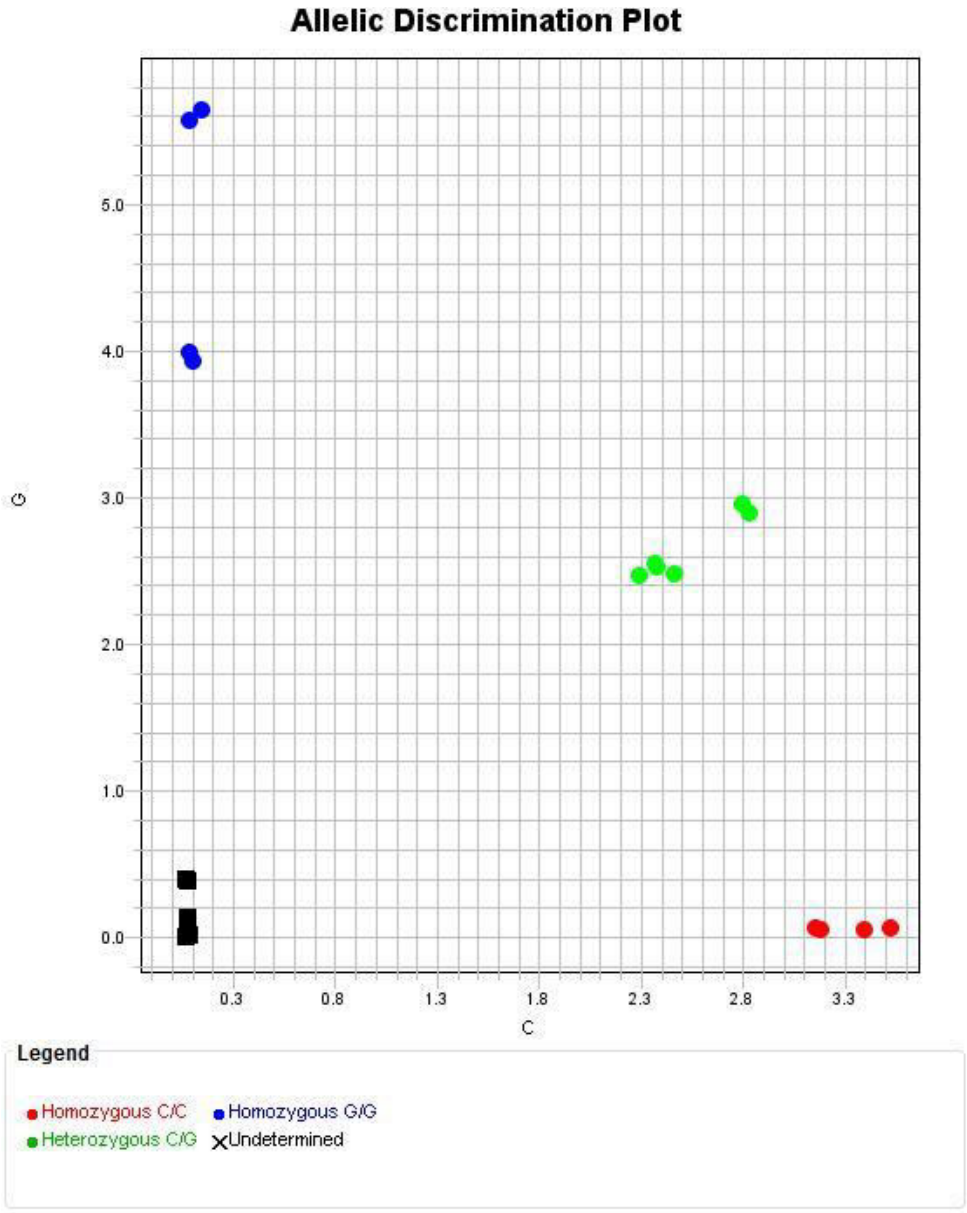


Figure 2: Allelic Discrimination Plot (IL-6 SNP). Each blue, green, or red dot represents an individual sample. Black dots represent No-template controls (NTC).

TaqMan® SNP Genotyping - Laboratory procedure

For every **SNP** analyzed, the reaction mix is prepared in a 0.5 ml reaction tube. The reaction mix consists of a universal ready-made TaqMan® *Genotyping Master Mix*, and a specific *Assay Working Stock* (containing the allele-specific probes, primers, and DNA polymerase). Every sample is run in duplicates, with two NTCs for every SNP. To compensate for possible pipetting losses, an excess of 20% should be calculated in. To mix properly, the reaction mix is then vortexed, followed by a brief centrifugation step for sedimentation. Every group prepares ***two reaction mix tubes*** (one for the IL-6 SNP, and one for the TNF-α SNP, respectively). Please follow pipetting in Table 1.

Table 1: Reaction mix TaqMan® SNP Genotyping

Component	Amount [µl] (17X run)
2X TaqMan® Genotyping Master Mix	210 µl
20X TaqMan Genotyping Assay Stock	21 µl

In the next step, the gDNA is diluted. The reaction setup is able to deal with appr. **1-20 ng DNA** per reaction. Hence, the previously isolated and quantified gDNA (Maxwell 16®, Nanodrop®, see before) has to be diluted in nuclease-free water in a way, in which all analyzed samples have the same concentration **of *1 ng/µl in a total volume of 100 µl* (see Table 2). Then, 11.25 µl** of the gDNA dilution are pipetted into the wells of a MicroAmp™ Optical 48-Well Reaction Plate (every sample is pipetted in duplicates, with two NTCs for every SNP), for the NTCs, 11.25 µl of nuclease-free water is pipetted instead. Please follow pipetting in Table 3.

Table 2: Preparation of the DNA dilution.

Group name			Diluted DNA [1ng/µl] / total: 100µl	
	DNA concentration [ng/µl]	Dilution factor	Amount DNA [µl]	Amount H20 [µl]
Sample #1				
Sample #2				
Sample #3				
Sample #4				
Sample #5				
Sample #6				
Sample #7				

Table 3: Pippeting schematic for the qPCR run. Reactions for the IL-6 SNPs (yellow highlight) and TNF-α SNP (blue highlight) are run on the same plate in duplicates, with two NTCs each. All samples of each group are loaded onto the same plate.

	1	2	3	4	5	6	7	8
A	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	NTC IL-6
B	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	NTC IL-6
C								
D								
E	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	NTC TNF-α
F	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	NTC TNF-α

Then, **13.75 µl** of the reaction mix (Table 1) for the respective SNP (IL-6: yellow, TNF-α: blue) is added into each well, adding up to a final volume of **25 µl** per well.

After completion, the plate is sealed with an adhesive cover, briefly vortexed, then centrifuged, and loaded onto the qPCR Thermal Cycler, undergoing the following amplification program (see Table 4).

Table 4: qPCR cycler program for TaqMan® SNP Genotyping

Step	Temperature	Length	Cycles
Polymerase activation	95°C	10 minutes	1 (Hold)
Denaturation	95°C	15 seconds	40 (cycling)
Annealing/extension	60°C	60 seconds	

The program determines the fluorescence signal after every cycle. It also consists of a pre- and post-PCR plate read, in which the fluorescent signals are determined twice (before and after the qPCR run). Based on this, the software generates the allelic discrimination scatter plot. After the run is completed, the 48-well plate can be opened carefully. The wells contain the amplified DNA fragment containing the SNP of interest, which can be loaded onto an agarose gel (see next step).

Agarose gel electrophoresis (FlashGel® DNA)

Agarose gel electrophoresis can be used to separate DNA fragments, based on their size and conformation. The underlying mechanism is the migration of DNA in an electric field. The phosphate backbone of nucleic acids leads to a negative charge. When exposed to an electric field, DNA will therefore migrate to the anode. Since DNA has an identical mass/charge ratio, the distance traveled of the molecules is inversely proportional to their size (linear DNA). (Lee P.Y., 2012).

Agarose is a linear, uncharged polysaccharide, which interacts with nucleic acids and proteins only marginally. After gelation, agarose polymerizes through non-covalent binding and a network of double helices is formed. Pores with the size of 100 - 300 nm are formed, where the size is determined by the amount of agarose used (Brown T.A., 1998). In our experiment, ready-made 2.2 % agarose gels are used, and **5.5 µl of each sample are mixed with 1.5 µl blue loading dye**, and then transferred onto the gel (total volume: 7 µl).

One lane is loaded with 4 µl of Marker (Lonza FlashGel® DNA marker). The run is started at 120 V for about 20 minutes. Figure 3 shows the final result.

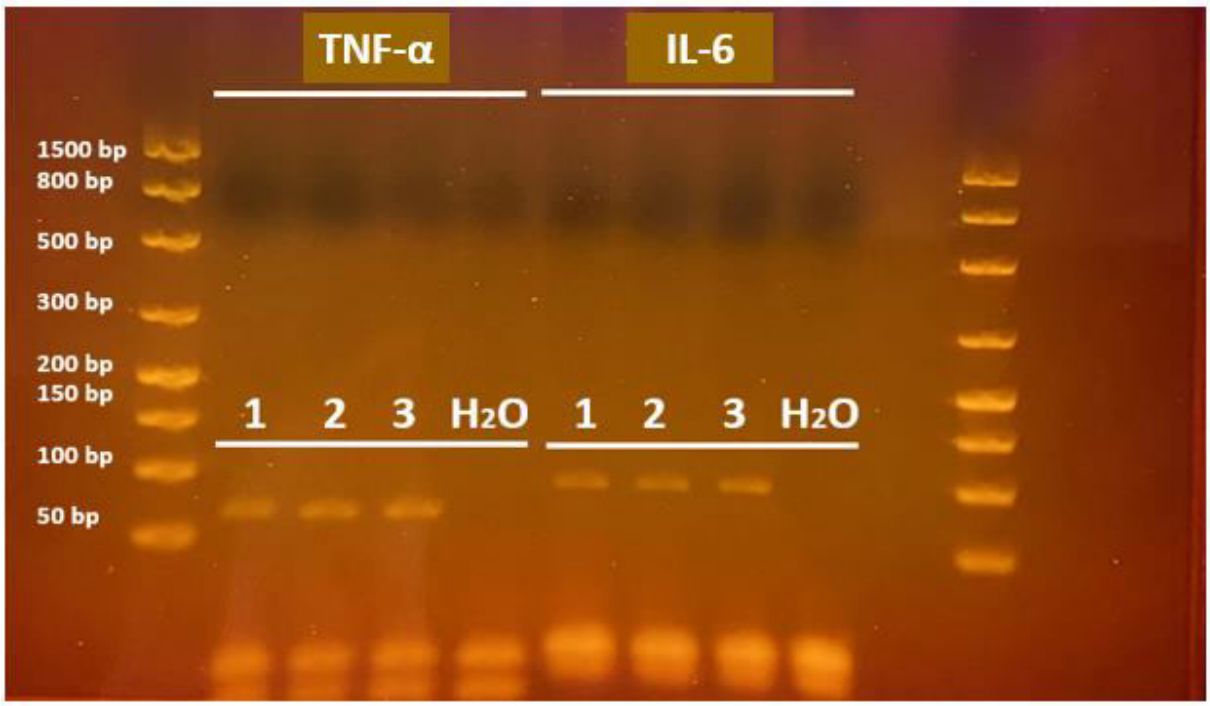


Figure 3: Typical Flash gel of amplified DNA fragments generated by **TNF-α** / **IL-6** SNP sequence analysis to detect the SNPs rs1800629 (-308G/A TNF-α) and rs1800795 (-174C/G IL-6), respectively. Lane#1 and #11 have been loaded with the DNA ladder as a size marker.