POLITECNICO DI TORINO

Master's Degree Thesis in Biomedical Engineering

Robotization of Cross Match test in Immunohematology

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Summary

The idea of this thesis project arises from the need for the robotization of the cross-match setup which would lead to an organizational, economic and safety benefit for the Immunogenetic area. Nowadays this test takes place manually in immunohematology laboratories and the automation of the test would make the co-presence of two people no longer necessary with the consequent reduction of costs and the error probability.

Cross match test or cross matching includes several steps, before a transfusion, and consists of testing if the donor's blood is compatible with the blood of the receiving patients and to determine compatibility in organ transplantation. The success of the test is useful for reducing immunological rejections that may cause serious consequences.

The first step necessary for the thesis work was to examine the entire procedure of the compatibility test to understand each step and to analyze human errors that can arise. One of the reasons that made automation necessary is in fact to reduce significantly the human error that in manual procedures are inevitable. In this phase, the support of Drs Silvia Deaglio and Drs Cristiana Caorsi of the Immunogenetics Laboratory of Molinette hospital was fundamental.

The idea was to develop a dispenser that would automatically withdraw the reagents from the Eppendorf tubes, positioned by the laboratory technicians, and dispense the desired quantities into the specific wells of the reaction plate according to a well-defined scheme.

After these meetings, a more detailed study of the test, a first setup of a working sequence and the prediction of the future robot's movements through the definition of flow charts were carried out autonomously.

For the concretization of the project, Inpeco SpA was available to collaborate for the realization of the device. It is important to underline that in the current Inpeco production there is no automatic system capable of realizing the cross-match procedure, carried out manually today, but there are technologies and skills that can be made available for our purpose. The objective of this visit was precisely to offer a starting point towards the possibility of reproducing some elements already present on the market and towards the knowledge of the limits of these elements that prohibit their use in the future robot object of the thesis.

Starting from an Inpeco device, that seemed to be the closest to our application, the possibility of modifying the aspiration/dispensing process was studied to make it usable in the automated procedure of the cross-match test. In this direction, starting from the study of the micropump that Inpeco's module uses, it was necessary to check the market in order to find a similar pump but with performances compatible with the test to be automated. The most important characteristics to be improved were pipetting precision and accuracy. The intention was to obtain a level of accuracy at least equal to that of the devices currently used in the laboratory.

The next step was to consider the dynamic of the future robot. Inpeco uses a 2-axes robotic arm and, to reproduce as much as possible the dispending dynamic of cross match test, it was necessary to add the third degree of freedom to the system. The best solution was to entrust the third axis to the microplate itself. For this reason, different companies were considered to look for a linear stage suitable for this application.

The last question to be resolved concerned the choice of the tip to use. Nowadays Inpeco makes use of robotic tips with a volume range too high for the cross-matching dispensation. For our intent, it was necessary to find a tip with an adequate volume range for the small quantities of interest and which, at the same time, guaranteed to dispense, with a single withdrawal, several reagents in several wells of the reaction plate.

All these components later were assembled in a single system to propose a concrete solution for the robotization of the cross-match test. Every step of the process was supported by Eng. Francesco Parodi of the Inpeco group who, thanks to his experience in the field of clinical automation, made the device subject of the thesis realizable.

1. Introduction

1.1 Joint project POLITO – D.O.T FOUNDATION

The non-profit DOT Foundation was established in 2017 by the Piedmont Region, the City of Turin, the AOU City of Health and Science of Turin, the University of Turin and the Polytechnic of Turin, to promote the culture of organ donation and transplantation.

Piedmont is historically a state-of-the-art region for organ donation and transplants. In fact, 2019 records the second-best performance ever by the number of interventions, with 3813 organ transplants in Italy, 419 of which in Piedmont.

The National Transplant Network is one of the clinical networks of our National Health System with the aim of promoting more efficient management of donation activities and improving the quality and safety of clinical, organizational and management processes.

In this intricate context, the adverse events monitoring aspect is decisive for making each phase of the process safe. In this direction, DOT Foundation supports the initiatives for the allocation of funds for the development of more efficient therapeutic practices related to transplantation.

Thanks to the collaboration of the Polytechnic of Turin with the Foundation, it was possible to study the cross-match test, one of the most important compatibility tests of organs, tissues or cells between donors and recipients.

1.2 Purpose of the thesis

The idea of this project comes from the need for the robotization of the cross-match setup which would lead to an organizational, economic and safety benefit for the immunogenetic area.

Nowadays this test takes place manually in immunohematology laboratories. The automation of the test would make the co-presence of two people no longer necessary with the consequent reduction of costs and the error probability.

For the realization of a system that satisfies these needs, it should be provided a manipulator robot and suitable sensors to control its motion. This thesis aims to create an automatic dispensing system that takes reagents from the Eppendorf tubes, positioned by the laboratory technicians, and dispense the desired quantities into the specific wells of the reaction plate according to a well-defined scheme.

This robot will be described in detail in the following chapters.

1.3 Organization of work

The thesis is organized as follows.

Chapter 2 illustrates the importance of automation in the diagnostic routine of the immunogenetics laboratory and the state of the art in this field. After a brief description of the advantages and benefits of the robotization of the clinical tests, the main existing liquid handling robotic workstations on the international scene are shown.

Chapter 3 presents the entire procedure to be automated starting from an introduction of the biological processes at the base of the cross match test and continuing with a detailed description of each step carried out manually today by the laboratory technician from the preparation of the reagents to the data processing phase.

In chapter 4 the different steps that led to the implementation of the complete system are addressed. This chapter initially illustrates the device from which the project started thanks to the collaboration with the Inpeco group. Then the various constituent elements of the cartesian manipulator are described also proposing different solutions.

In chapter 5 the steps of the dispensing process of the liquid handling are reported. This chapter presents the different aspiration phases from initial air gap aspiration to biological liquids withdrawal and transport air gap suction necessary to ensure maximum precision and accuracy in the dispensing of volumes at the microliter level.

Chapter 6 proceeds with the description of the capacitive monitoring system of the pipetting arm useful to ensure the aspiration and dispensing of the correct volumes of reagents without compromising the success of the test. Liquid level and clot detection system are then illustrated.

Each time the pipettor arm needs to change the reagent to be dispensed, the tip must be replaced with a new one. This tip loading/unloading process takes place in the so-called tip loader. This process is summarized in chapter 7.

The last step of this project concerns the assembly of all the elements considered. Chapter 8 describes the 3D model of the device with an evaluation of the weight and dimensions followed by an estimate of the cost of the corresponding device.

The last chapter concerns the dispensing algorithm of the new robot describing in detail each phase of the process including also the manual operations that the operator should perform.

2. Automation in Immunohematology

2.1 Purposes of robotization

Automation finds applications in the immunohematology field where any human errors can bring significant consequences for patients. Automation provides the advantage of improving the quality of testing by:

- Reducing the risk of human error compared to manual performance relating to sample identification, reagent selection, test execution, interpretation of results and their transcription;
- Improving traceability of all elements which must be archived, accessible and usable at any time;
- Improving objectivity and reproducibility of results of immunohematology tests;
- Controlling all malfunction's alarms allowing the operator to intervene in any phase of the procedure;

Exists several automated equipment available at present from fully-automated systems, able to achieve the result without any manipulation of the samples, to semi-automated machines that require an operator for the centrifugation, incubation or washing phases.

The decision to buy an automated system depends on the type of services provided, costs, space availability and staff competency. The table below shows the main advantages and disadvantages of the two categories of automation.

	ADVANTAGES	DISADVANTAGES
FULLY-AUTOMATED SYSTEMS	- Improvement in objectivity and reproducibility of results - Test execution time reduced - Improvement traceability of all elements - 'Walk Away' → possibility of performing other activities during tests - Standardization of procedure	- High cost and large initial investment
SEMI-AUTOMATED SYSTEMS	- Low cost	- Manual Input - Low standardization test - Less complete traceability

Table 1: Fully vs Semi-Automated systems

In view of the above, semi-automated systems may be appropriate for a small workload, however, for transfusion centers with a substantial number of tests to be performed at the same time, a fully-automated system is always a better option. Either way, it is necessary to insist on adequate staff training which makes the laboratory technicians comfortable with the new technology. In fact, whatever the improvement is, none of the automated systems allows solving all the technical difficulties present in immunohematology.

Adopting automation in immunohematology is expected to bring about major changes in the pretransfusion testing laboratories and an overall facelift in the working of a transfusion service setup. Continuous scientific progress coupled with automation and computerization is the future requirement for maintaining the highest level of quality in the blood bank.

The safety of blood and blood components is the major concern of every blood center. Blood center needs quality systems and state of the art infrastructure to maintain good laboratory practices despite the heavy workload. The Immunohematology laboratory is responsible for supplying compatible blood through pretransfusion compatibility testing performed in order to prevent the transfusion of incompatible donor red cells that might result in an immune-mediated hemolytic transfusion reaction. To prevent manual errors, the automation of blood transfusion serology can prove to be a valuable tool in this laboratory.

The ABO and Rh blood grouping and the crossmatching between lymphocytes' donor and the recipient's serum are integral steps of the compatibility testing. The compatibility test that this work proposes to automate is cross match test.

2.2 State of the art

Automation in Blood bank serology was introduced in the developed countries in the 1960s. Since those times it was known that robotic transfusion testing improves patient safety by reducing human error, requires less manpower than manual tube testing and could help save on staff costs. On top of this, it is important to point out that an automated device is also much easier to teach than manual test tube tests.

For this reason, automation in immunohematology has been adopted by more and more centers rapidly becoming a standard testing technology in developed nations.

Nowadays, different manufacturers provide different types of liquid handling devices from fully automated to semi-automated immunohematology platforms. All these systems share the same basic architecture that can be seen in the figure below.

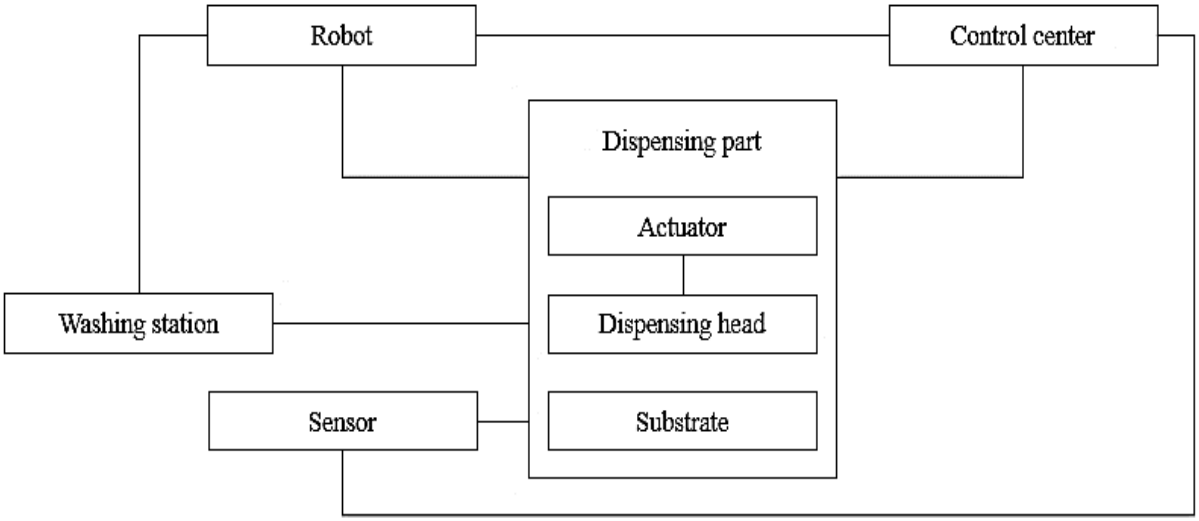


Figure 1: Generic architecture of liquid handling robotic system

The control center manages the robotic arm strokes through actuators or other organs capable of generating movements between the dispensing part and the washing station used to clean the dispensing head and avoid cross-contamination. The dispensing head dispenses biological liquids and reagents on the substrate represented by empty tubes or well microplates that will be analyzed in the subsequent phases. Sensors are responsible for returning feedback analyzed by the control center and are essential to ensure maximum accuracy and precision in the dispensing phase.

In addition to these basic elements, liquid handling devices can provide other functionality based on the degree of automation.

The selection of better equipment for a blood center depends on the workload, resources, and space available. The main automatic systems available today are:

1. Bio-Rad - IH-1000
2. Diagast - QWALYS 3
3. Ortho Clinical Diagnostics - ORTHO AutoVue Innova
4. Immucor - NEO Iris
5. Grifols – WADIANA

2.2.1 Bio-Rad - IH-1000

The IH-1000 is a fully automated workstation produced by Bio-Rad (Switzerland) which can be used to execute a great variety of immunohematological test procedures.

Figure 2: IH -1000 (Bio-Rad)

The IH-1000 is designed for automated blood grouping determination using IH-Cards, utilizing hemagglutination and gel filtration as the principle of operation. The instrument is intended for detection of ABO blood group and Rh antigen typing, antibody screening and identification, direct antiglobulin test and AHG cross match.

2.2.2 Diagast - QWALYS 3

The QWALYS 3 is a fully-automated and high-throughput system for immunohematology produced by Diagast (France). In this system grouping, phenotyping, donor antibody screening, identification and cross matching can all be performed using nanotechnology based on the magnetization of red blood cells.

Figure 3: Qwalys 3 (Diagast)

Qwalys 3 is a blood matching automated system with complete barcode management and with a loading capacity of 160 samples. This system includes all steps from the dispensing of the samples and reagents to the interpretation of results.

2.2.3 Ortho Clinical Diagnostics - ORTHO AutoVue Innova

The ORTHO AutoVue Innova is a fully automated system produced by Ortho Clinical Diagnostics (USA). This system provides automated test editing functions such as liquid pipetting, cassette handling, incubation, centrifugation, reaction grading and interpretation until data management.

Figure 4: ORTHO AutoVue Innova (Ortho Clinical Diagnostic)

This workstation can be used to execute different blood tests from blood grouping to antibody screening/identification and cross matching using the column agglutination technique.

2.2.4 Immucor (USA) - NEO Iris

NEO Iris is another possible solution for large transfusion medicine laboratories and blood banks produced by Immucor (USA).

Figure 5: NEO Iris (Immucor)

In this system different modules can dispense, incubate, centrifuge and read simultaneously providing maximum operational efficiency. NEO Iris helps labs to automate more of their workload through continuous processes that allow reproducing several hundred tests per hour.

2.2.5 Grifols - WADIANA

The WADIANA is a fully automated compact instrument manufactured by Grifols (Singapore). This system can identify samples, dispensing reagents, incubate them in two independent blocks, centrifuge and report results. It is possible to replicate tests such as blood group, antibody screening/identification, enzymatic test and cross match through column agglutination technology.

Figure 6: WADIANA (Grifols)

2.3 Requirements for the cross match robotization

Despite the benefits of the newer devices and automation and although there are innovative technologies on market seen previously, many transfusion centers and immunogenetics laboratories still use manual methods for routine blood group determinations and antibody screening tests. The current dearth of automation in this field is one of the reasons why some pre-transfusion tests continue to have a high error potential.

For automation to be successful, it must fit the needs determined by the immunogenetics laboratory interested. In the case subject of the thesis, the devices available on the market do not fully meet the needs of the Immunogenetics Laboratory of Molinette hospital in Turin. According to this lab, there are different reasons shown below why it is necessary to design a new automatic dispensing device suitable for cross match test.

1. All available devices consist of different steps from the reading of the sample to the analysis of the results. Molinette's lab requires automation of the only dispensing phase because it is already equipped with bench centrifuges, incubators and a data processing system. The dispensing phase is the most critical in the entire cross match procedure and the most exposed to human errors. This step is very operator dependent and variation is inherent in any one operator's technique and among multiple operators.

2. The continuous loading of samples and reagents, the versatility of use combined with the walk-away philosophy and high productivity make blood matching automated systems particularly suitable for the management of medium-large daily routine of examinations. The Molinette's laboratory carries out the cross-match test only when an organ donor is present and therefore only in this eventuality. For this reason, a device of those present today is not compatible.

3. The robotic workstations for liquid handling on the market do not reach the accuracy imposed by the cross-match test. In Molinette's laboratory, having to dispense volumes of biological liquids at the microliter level, there is a need for greater precision.

4. Automation is too expensive for Immunohematology laboratories and for this reason, although the fully-automated devices are very attractive, they tend to go towards cheaper solutions.

3. Cross Match

3.1 Transplant Immunology

Transplantation is the process of moving cells, tissues, or organs, from one site to another, either within the same person or between a donor and a recipient. There are several types of transplantation involving tissues and organs:

- **Autografts** are grafts transferred from the same individual.
- **Allografts** are grafts transferred from a donor to a non-genetically identical individual of the same species.
- **Xenografts** are grafts between two different species.

Allografts are the most common type of transplant. Recognition of transplanted cells as self or non-self is determined by the histocompatibility genes, which differ among individuals of the same species. The mainly responsible for the violent and rapid reactions of rejection is the major histocompatibility complex (MHC).

The human leukocyte antigen (HLA) system is a group of related proteins that are encoded by the major histocompatibility complex (MHC) in humans. These membrane glycoproteins are responsible for the activation of the immune response.

The molecules of the major histocompatibility complex that present the antigen are divided into two main classes:

- Class I HLA is expressed by all nucleated cells;
- Class II HLA is expressed by cells that present the antigen: monocytes and macrophages, dendritic cells, B lymphocytes and some types of T cells;

If a transplanted tissue does not have the same HLA molecules as the recipient (HLA-incompatible), the graft is destroyed by the reactivity immune system (rejection). For this

reason, before performing a transplant it is necessary to ensure that the donor and the recipient have some if not all HLA molecules equal.

3.1.1 Pathways of antigen processing

The main function of HLA molecules is the presentation of peptides to the cells of the immune system. Depending on the type of molecule (class I or class II), the role it plays is different.

- *Class II MHC pathway*
  Antigen-presenting cells (APC) can endocyte microbial proteins through different mechanisms and fuse with lysosomes forming phagolysosomes. Within these vesicles, proteins are fragmented from proteolytic enzymes into small peptides of various lengths and sequences. APCs constantly synthesize MHC-class II molecules in the endoplasmic reticulum and each molecule carries a protein called 'invariant chain' that contains a sequence called CLIP which binds to the pocket of the class II molecule making it inaccessible. Class II molecules are directed towards the cell membrane, but, during their displacement, they merge with phagolysosomes. If a class II molecule finds a peptide that adapts and the invariant chain breaks, the association takes place and this complex reaches the cell surface, otherwise the MHC molecule is degraded by proteolytic enzymes.

- *Class I MHC pathway*
  Antigenic proteins can also be produced directly into the cytoplasm from viruses that lodge in cells infected. All these proteins are loaded in cylindrical proteolytic organelles called proteasomes. Proteins are cut with a catalytic mechanism in fragments of variable length and sequences that can be mounted on MHC class I molecules, synthesized by the endoplasmic reticulum. Peptide fragments are displaced within the endoplasmic reticulum from a protein defined TAP (Transporter Associated with Antigen Processing). The newly synthesized MHC class I molecules can bind these peptides if they are suitable for binding to the pocket polymorphic present in their structure. If the molecule doesn't find a peptide to bind, the molecule is degraded, otherwise continues its journey towards the cell surface.

3.1.2 Rejection

Antibodies, or immunoglobulins (Ig), are proteins produced by some white blood cells, the B lymphocytes, which are involved in the immune response against any or recognized as dangerous. Once the antibodies come into contact with specific "foreign" molecules, called

antigens, they can interfere with the ability of pathogenic microorganisms to interact with the cells of the organism or to stimulate their elimination by specific immune cells.

Among the myriad of antibodies present, the IgM and IgG antibodies are the body's main defense weapons. IgMs are the first antibodies to be produced after contact with a foreign agent providing short-term protection. The IgM concentration increases for a few weeks and then decreases when IgG production begins.

IgG concentration generally increases a few weeks after infection and gradually decreases until it stabilizes. IgG helps to develop a secondary immune response which occurs on subsequent exposures to the same antigen. They represent the "memory" of the immune system recognizing microorganisms with which it has already come into contact.

Allogenic identification can involve different antibodies which, recognizing the transplant antigens, bring to rejection. Transplant rejection is classified according to histopathological and kinetic characteristics in hyperacute, acute and chronic:

- **Hyperacute rejection** appears immediately after transplantation. It is caused by accidental ABO blood type incompatibility.
- **Acute rejection** occurs between one week and several months. It is caused by an immune response directed against the graft.
- **Chronic rejection** occurs months or years after organ or tissue transplantation. It is caused by T-cells mediated process resulting from the foreign MHC “looking like” a self MHC carrying an antigen.

3.2 Cross Match test

One of the main clinical tests used for reducing immunological rejection is the cross-match test. Cross matching is an antigen-antibody reaction through which we can detect the clinically significant antibodies, IgG and IgM, mostly present in recipient serum which react with an antigen on donor red cells.

This compatibility test consists of two phases during which laboratory technicians take care to prepare reagents which will be dispensed on a plate. For the first phase five tubes are prepared with:

- Negative control
- IgG positive control
- IgM positive control
- Recipient patient’s serum

- Donor’s cells (lymphocytes)

For the second phase two tubes are prepared with two mixture of antibodies:

- IgG fluorescent antibodies
- IgM fluorescent antibodies

Between phase 1 and phase 2 the laboratory technician takes the plate and initially proceeds with 20 min incubation period at 22 °C, and then with 2 PBS solution washes. After the second phase, a 10 min incubation period at 22°C and 1 PBS solution wash follow.

3.2.1 Materials

During the procedure, different elements are used. Reagent prepared by technicians are stored inside Eppendorf tube having the following characteristics:

Volume	1,5 ml
Height	38 mm
Bottom type	conical
Outer diameter	12 mm
Inner diameter	10 mm

Table 2: Eppendorf tube description

To take biological fluids from Eppendorf tubes are used different air interface Eppendorf pipettes depending on the volume to be withdrawn. These pipettes are used by inserting the inferior end into an appropriate tip.

These pipettes work through the air displacement driven by a piston. A vacuum is generated by the vertical displacement of metal or ceramic piston inside a hermetic sleeve. When the piston moves upwards, guided by the depression of the piston, a vacuum is created in the space left free by the piston. The air from the tip rises to fill the space and the air from the tip is then

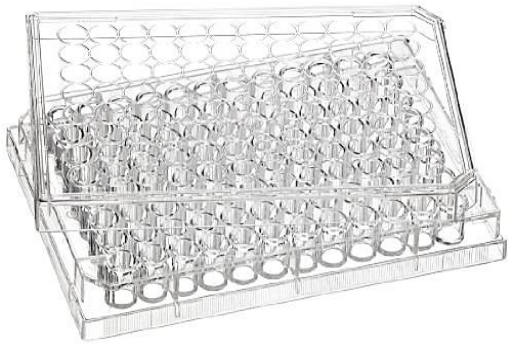
replaced by the liquid, which is drawn into the tip and then available for transport and dispensing elsewhere.

Operating mode	Manual
Type of pipetting	Air Interface
Volume range	Depends on type
Number of channels	Single-channel

Maximum tolerated errors (ISO 8655)				
Nominal volume	**systematic**		**random**	
	±%	**±µL**	**±%**	**±µL**
1 µL	5,0	0,05	5,0	0,05
5 µL	2,5	0,125	1,5	0,075
10 µL	1,2	0,12	0,8	0,08
20 µL	1,0	0,2	0,5	0,1
50 µL	1,0	0,5	0,4	0,2

Table 3: Eppendorf pipettes description

The cross-reaction takes place in 96-wells U-shaped-bottom microplates:



Capacity well	0,32 ml
Diameter well	6,42 mm
Length	127,61 mm
Width	85,3 mm

Table 4: Falcon 96-wells microplate description

3.2.2 Plate scheme

In order to set up the microplate, it is necessary to use the so-called "plate scheme" which explains in detail where to dispense the individual reagents.

96 Wells	IgG						IgM					
	1	2	3	4	5	6	7	8	9	10	11	12
A	NEG	S3	S7	S11	S15	S19	NEG	S3	S7	S11	S15	S19
B	NEG	S3	S7	S11	S15	S19	NEG	S3	S7	S11	S15	S19
C	POS IgG	S4	S8	S12	S16	S20	POS IgM	S4	S8	S12	S16	S20
D	POS IgG	S4	S8	S12	S16	S20	POS IgM	S4	S8	S12	S16	S20
E	S1	S5	S9	S13	S17	S21	S1	S5	S9	S13	S17	S21
F	S1	S5	S9	S13	S17	S21	S1	S5	S9	S13	S17	S21
G	S2	S6	S10	S14	S18	S22	S2	S6	S10	S14	S18	S22
H	S2	S6	S10	S14	S18	S22	S2	S6	S10	S14	S18	S22

Figure 7: Plate scheme

In this configuration are indicated negative control (NEG), IgG positive control (POS IgG), IgM Positive control and the serum corresponding to the receiving patient X (SX).

In each plate, cross match reaction takes place between only one donor and different recipient patients. Each serum is always tested simultaneously in two different wells, in fact, as can be seen by Fig. 4, there are always 2 wells dedicated to the same serum and this means that the same test is always repeated twice. In this way, if the test fails in one of the wells, the test can happen in the other well.

The dispensing of the left half of the plate also occurs equally in the right half in the first phase of the cross match. Instead, what changes between the two parts is the type of fluorescent antibody that is added during the second phase of the test: IgG fluorescent Ab for the left half and IgM fluorescent Ab for the right half.

Following exactly this scheme, laboratory technicians must dispense the reagents through the Eppendorf pipettes described in the previous paragraph.

3.2.3 Dispensing phase

The dispensing phase is the most critical step during cross match procedure due to the operator's high risk of making mistakes. An error at this stage can compromise the entire success of the test. During the dispensation, the co-presence of two technicians is required to reduce human errors as much as possible.

The different steps will be described below if only a donor and a single recipient (S1) are available. In this case, cross match test will take place in the first six wells of the first column.

1) Donor cells dispensation: the technician takes the Eppendorf pipette, inserts the appropriate tip, withdraws 15µL from the Eppendorf tube with donor cells and dispenses 15µL in A1 well. These passages are repeated for all six wells. The tip is changed only after all six wells have been filled.

2) Negative control dispensation: the technician takes the Eppendorf pipette, inserts the appropriate tip, withdraws 15µL from the Eppendorf tube with negative control and dispenses 15µL in A1 well. These passages are always repeated also for B1, A7 and B7 wells. The tip is changed after each dispensing to avoid contamination between one well and another.

3) IgG positive control dispensation: the technician takes the Eppendorf pipette, inserts the appropriate tip, withdraws 15µL from the Eppendorf tube with IgG positive control and dispenses 15µL in C1. These passages are always repeated also for D1 well. The tip is changed after each dispensing to avoid contamination between one well and another.

4) IgM positive control dispensation: the technician takes the Eppendorf pipette, inserts the appropriate tip, withdraws 5µL from the Eppendorf tube with IgM positive control and dispenses 5µL in C7. These passages are always repeated also for D7 well. The tip is changed after each dispensing to avoid contamination between one well and another.
5) S1 dispensation: the technician takes the Eppendorf pipette, inserts the appropriate tip, withdraws 15µL from the Eppendorf tube with the recipient serum and dispenses 15µL in E1. These passages are always repeated also for F1, E7 and F7 wells. The tip is changed after each dispensing to avoid contamination between one well and another.

6) Incubation period (20 min/22°C) + 2 PBS solution washes

7) IgG fluorescent antibodies dispensation: the technician takes the Eppendorf pipette, inserts the appropriate tip, withdraws 40µL from the Eppendorf tube with IgG fluorescent antibodies and dispenses 40µL in all six wells on the left half of the

microplate. The tip is changed after each dispensing to avoid contamination between one well and another.

8) IgM fluorescent antibodies dispensation: the technician takes the Eppendorf pipette, inserts the appropriate tip, withdraws 40µL from the Eppendorf tube with IgM fluorescent antibodies and dispenses 40µL in all six wells on the right half of the microplate. The tip is changed after each dispensing to avoid contamination between one well and another.

9) Incubation period (10 min/22°C) + 1 PBS solution washes

3.2.4 Data Analysis

After the dispensing phase, there is the final step of results reading through a flow cytometer which will give a negative or positive output for each well. In the negative and positive control wells the system should return respectively negative and positive results if the entire procedure has been correctly performed. For all other wells the system will output the XM result:

- Negative Patient → No Immunocomplex formation → Compatible → Donor is immunologically suitable for that patient

- Positive Patient → Yes Immunocomplex formation → Not Compatible → Donor is not immunologically suitable for that patient

4. Implementation of the system

4.1 Inpeco group collaboration

In this work the collaboration with the Inpeco company was fundamental. Inpeco SpA, located in Val della Torre (TO), Givoletto Street 15, is a multinational company in the automation field for clinical analysis laboratories. The philosophy that supports the group is the possibility of limiting human error in the healthcare sector through the automation of operational processes otherwise manually performed.

Inpeco has solutions both for the process inside the laboratory and for the sampling point and, among all its products, the Aliquoter Module seemed to be very close to the project in question. This module allows to take a biological sample from a primary tube and divide it into secondary tubes according to the configured test purpose. For this reason, the robotic arm of the aliquoting module was taken as a starting point for the automation of the cross-match test.

4.2 Inpeco pipetting arm

The Inpeco robotic arm is a constituent element of an entire Tecan liquid handling system. Tecan workstation consists of one 8-pipettors arm and a tool arm with three degrees of freedom with the following robotic performance.

Feature	Specification
Robotic transfer speed	Approximately 4 seconds to take the stack and place it on the work surface, washer or reader
Robotic performance	Force in Z-direction: 30N Arm Force: 240N at the far end Z-travel range 150 mm

Table 5: Tecan robotic performance

Feature		Speed	Ramp
Pipetting arm	x-motion	750 mm/sec	1000 mm/sec^2
	y-motion	300 mm/sec	1500 mm/sec^2
	z-motion	350 mm/sec	800 mm/sec^2
Tool arm	x-motion	750 mm/sec	1000 mm/sec^2
	y-motion	350 mm/sec	1500 mm/sec^2
	z-motion	110 mm/sec	500 mm/sec^2
	G-motion	150 mm/sec	3000 mm/sec^2
	R-motion	400 degree/sec	1500 degree/sec^2

Table 6: Tecan motion speed

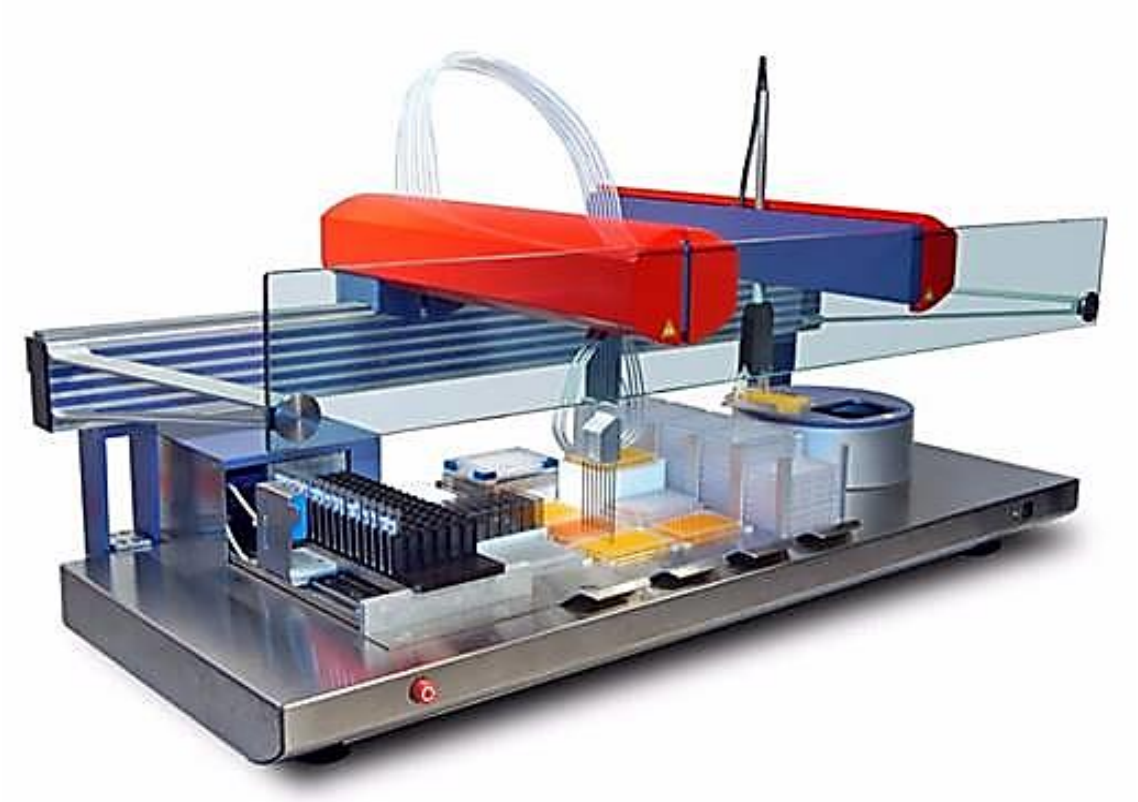


Figure 8: Tecan liquid handling system

Inpeco group buys only the dispensing arm without the x-axis reducing the system to only two degrees of freedom. Inpeco's 2-axis manipulator is equipped with one pipetting channel that, through the principle of positive displacement, transfers liquid volumes from source to destination thanks to an individual micro-annular gear pump. This robotic arm is the main element of the Inpeco Aliquoter module.

The pipetting operation of this module involves the aspiration of a portion of the sample from the primary tube and its dispensing in four daughter tubes. The micro-annular gear pump can dispense a volume range between 5 µl and 1000 µl with the following coefficients of variation:

	Volume	**CV**
Pipetting Performance	5 µl	< 10%
	50 µl	< 2%
	1000 µl	< 1%

Table 7: Performance Inpeco pump

The quantities to be dispensed during cross matching are of the order of a microliter, much lower than those treated by the Aliquoter module. For this reason, higher levels of precision than those listed above are required.

The objective was to use a similar pump but having levels of accuracy greater or equal than those of the devices used by laboratory technicians to dispense liquids into the plate wells.

In the immunogenetics laboratory, operators use the Eppendorf pipette for handling volumes of liquid in the microliter scale. These pipettes work thanks to a displacement of air driven by the piston. A vacuum is generated by the vertical stroke of a metal or ceramic piston inside a hermetic sleeve. When the piston moves upwards, guided by the depression of the piston, a vacuum is created in the space left free by the piston. The air from the tip rises to fill the space, and the air from the tip is then replaced by a liquid, which is drawn into the tip and then available for transport and dispensing elsewhere.

Eppendorf mechanical pipettes performance are shown in the table below.

	Volume	**CV**
Pipetting Performance	5 µl	2,5 %
	50 µl	1 %
	1000 µl	0,8 %

Table 8: Performance Eppendorf air interface pipette

The coefficient of variation is lower in the case of Eppendorf pipettes. The intent was precisely to make these two values at least equal.

To overcome this problem, it was necessary to look for a pump available on the market with accuracy and precision suitable for cross match test. This was the most important characteristic to be improved. For the realization of this pump, it was decided to collaborate with HNP Mikrosysteme which develops, manufactures and markets pumps that dose small quantities of liquids quickly and accurately all over the world.

4.3 HNP Mikrosysteme micropump

HNPM develops and produced micro annular gear pumps to reach highly precise metering volumes in the microliter to milliliter range and the smallest volumetric flows. Thanks to their indispensable technical support, HNP operators, helping us with our intent, chose for this application mzr-2921X1 pump of the low-pressure pump series.

Figure 9: mzr-2921XI HNP micropump

The compact micro annular gear pump mzr-2921X1 is an innovative pump concept for use in analytical instrumentation. The pump generates a low pulsation, highly precise flow featuring low shear stress. It dispenses at a high precision the lowest volumes in the microliter range. The compact design with a brushless motor as well as an integrated motion controller for speed and position control small dimensions are the main characteristics of this pump.

4.3.1 Operating principle of the micro annular gear pump

Micro annular gear pumps are positive displacement pumps. A positive displacement pump moves a fluid by repeatedly enclosing a fixed volume, with the aid of seals or valves, and moving it mechanically through the system. The pumping action is cyclic and can be driven by pistons, screws, gears, lobes, diaphragms or vanes. There are two main types: reciprocating and rotary.

The pump in question is of the rotary type and contains two rotors bearing slightly eccentrically to each other. An externally toothed internal rotor and an annular and an internally toothed external rotor. Due to their cycloid indenting, the rotors remain interlocked at any time, forming during rotation a system of several sealed pumping chambers.

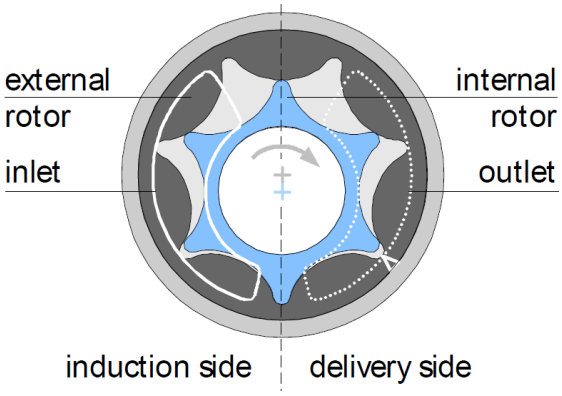


Figure 10: Principle of the micro annular gear pump

As the rotors revolve around their axis, the pumping chambers increase on the aspiration (suction) side and simultaneously decrease on the delivery side of the pump. A homogenous flow is generated between the kidney-like inlet and outlet.

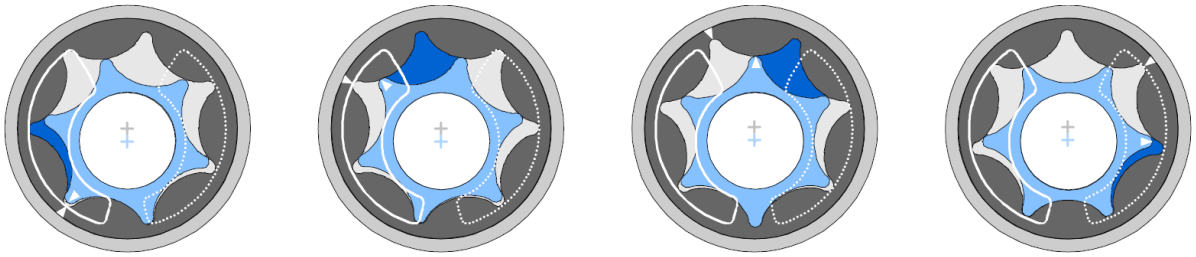


Figure 11: Operating principle of the micro annular gear pump

In the case of rotary displacement pumps, the delivered amount of liquid may be easily calculated from the displacement volume Vg of the pump and the number of revolutions of the rotor n. Displacement volume stands for the volume of liquid that is moved within one revolution cycle of the rotor. This relation is illustrated by the following formula.

$$Q = \eta_{Vol} \cdot V_g \cdot n$$

The volumetric efficiency η_{Vol} shows the relation between the actual and theoretical flow rate values. The existing differences result from the internal movement of the liquid during the operation. The gap between both rotors and between the rotors and the adjacent case parts lies in the range of a few micrometers. This precision is the key factor enabling to achieve volumetric efficiency close to 100%.

4.3.2 Construction

The micro annular gear pump is composed of the pump head, the drive unit (brushless DC-motor) and the connection cable with plug.

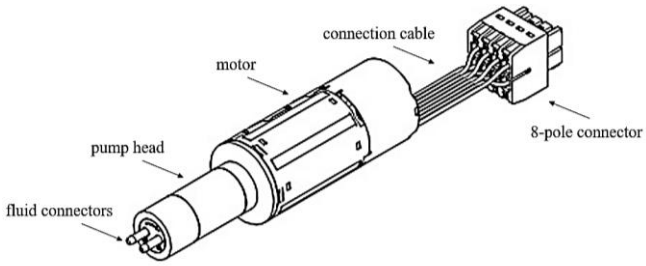


Figure 12: Layout of the micro annular gear pumps mzr-2921X1

4.3.3 Dosage precision

Thanks to the high dosage precision, the HNPM pump can achieve higher accuracies than the Eppendorf mechanical pipettes manually used in the laboratory today. This is confirmed by the red line in the following figure which shows the variation coefficient expressed in % in relation to the dispensed volume.

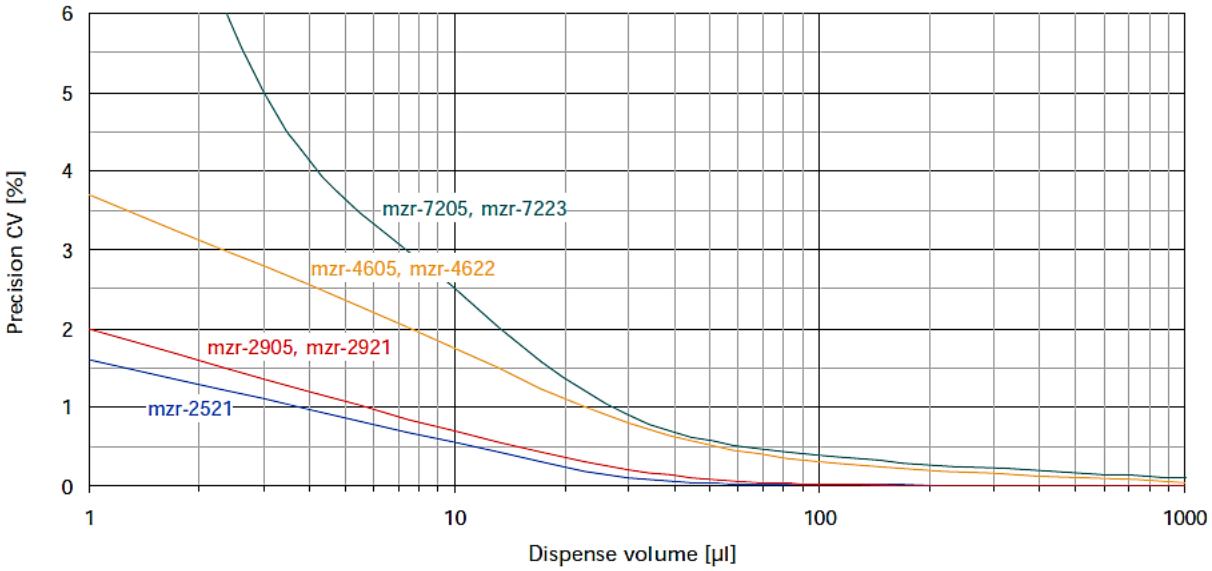


Figure 13: mzr-2921X1 dosage precision

Precision improves with increasing of the dispense volume up to an accuracy value of 100%. Unlike the Eppendorf mechanical pipette which presents a coefficient of variation of 2,5% for

a 5 µl dosage, the HNPM pump guarantees a much better performance with a coefficient of variation close to 1% for the same dosage.

4.4 Linear Guide

The robotic arm of Inpeco allows to travel only two cartesian directions (y and z). For the robotization of the cross-match test is necessary to cover also the third direction. Among the various possibilities, the least expensive and easiest to implement was obtained by entrusting the third degree of freedom to a linear guide capable of transporting the 96-well microplate along the direction that cannot be traveled by the end effector of the Inpeco arm.

The slider of interest must translate the plate to allow the robot to deposit the liquid in all the wells. For this reason, it must have a minimum stroke of 100 mm and a resolution equal to the distance between two adjacent wells.

Two different companies specialized in this field were considered. The first solution was proposed by the Ewellix company. The linear axis suggested is represented in the figure below.

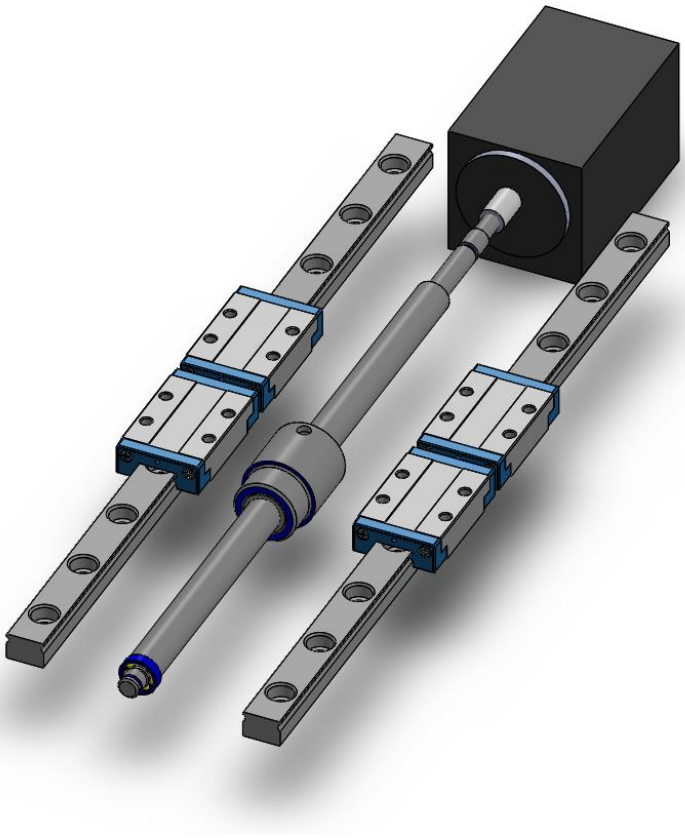


Figure 14: Ewellix first solution

This system consists of:

- two miniature profile rail guides 200mm long with 2 carriages per rail to give more stability to the plate;
- one 8mm nominal diameter rolled ball screw with a 150 mm threaded length;
- radial ball bearings with 8mm internal diameter;
- a stepper motor and a torsional joint to ensure correct motion transmission and to absorb sudden variations in torque or to lower the vibration frequencies of the transmission;

This solution additionally requires the realization of a support element fixed to the ball screw allowing the plate to translate. Thanks to this mechanism, it is possible to transform a rotary motion into a translational motion suitable for translating the microplate in the desired direction. For this proposal, Ewellix can only supply the two linear guides and the ball screw with some support bearings. For other components, it is necessary to contact other manufacturers.

The stepper motor is a pulsed direct current synchronous electric motor with brushless electronic management that can divide its rotation into many steps. This type of motor consists of many turns arranged in the form of a circle.

When current passes through one of the coils it becomes magnetic and this moves the motor shaft in its direction. The following figure explains this concept.

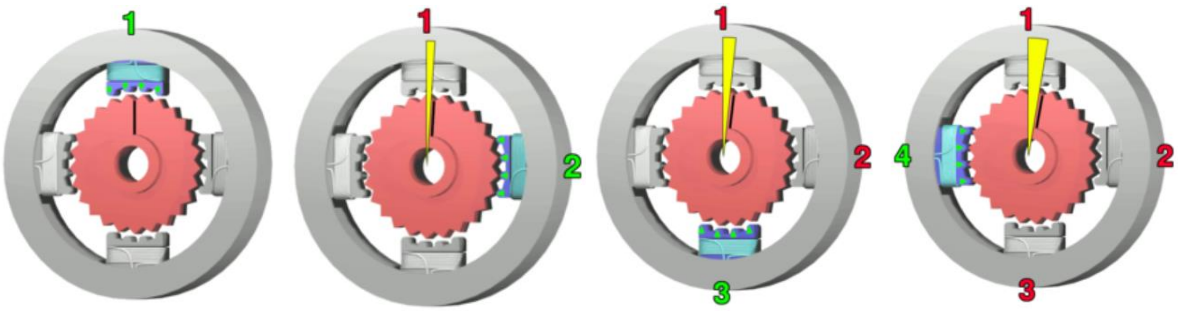


Figure 15: Stepper motor working

1. The top electromagnet (1) is energized, attracting the nearest tooth of a metal rotor-shaped tool. With the tooth aligned with electromagnet 1, rotation towards electromagnet 2 will begin;

2. The top electromagnet (1) is switched off and the right electromagnet (2) is energized, pulling the next tooth slightly to the right. This process produces a 3.6 ° rotation in this example;

3. The lower electromagnet (3) is excited and another 3.6 ° rotation occurs;

4. The electromagnet on the left (4) is excited, there is another rotation of 3.6 °. When the electromagnet at the top (1) is energized again, the spool teeth will have to rotate to the next position; since there are 25 teeth, it takes 100 steps to make a full rotation.

In addition to this possibility, the same company proposed a cheaper but less precise alternative. Instead of using a ball screw driven by a stepper motor, a linear actuator could be used to translate the plate that will slide on the profiled guides. Linear actuators are motorized mechanical cylinders that transform the rotary motion of the motor into the linear movement of the thrust tube.

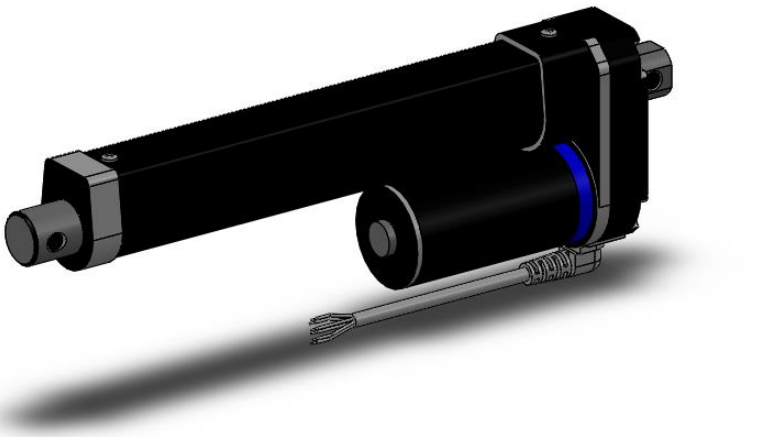


Figure 16: Ewellix linear actuator

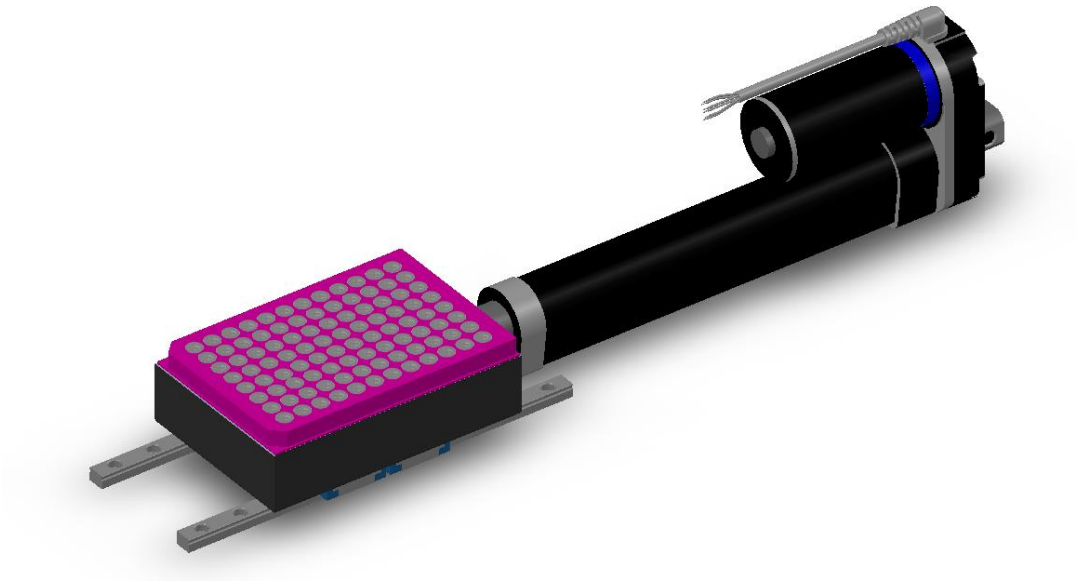


Figure 17: Ewellix second solution

These two solutions are the cheapest on the market but, in both two cases, after buying all the single components separately, it is necessary to reassemble them autonomously. This represents a negative aspect.

In addition to the Ewellix proposal, the possibility of having a complete motorized linear axis much easier to manage was considered. Newport company supplies several motorized linear stages with different configurations. The stepper motor version, among their products, resulted the cheaper solution compatible with this application.

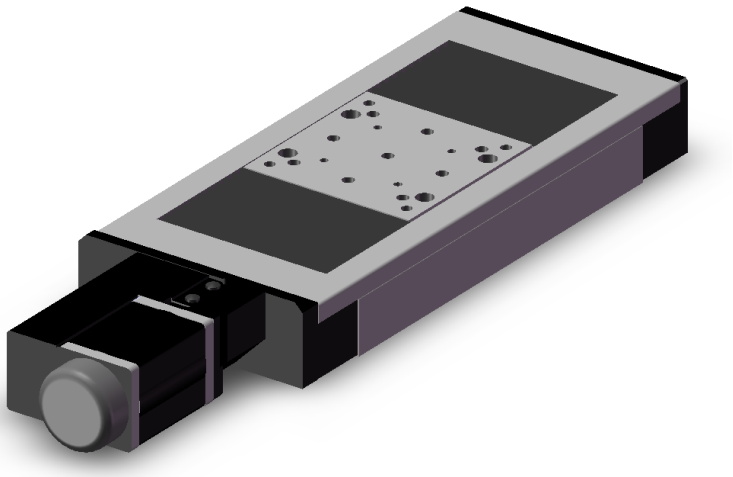


Figure 18: Newport linear slider

The device shown above, therefore, allows to obtain a complete motorized solution easy to manage with a 100mm stroke and high resolution but, in contrast, represents the most expensive solution compared to those seen up to now.

For all devices presented, it is necessary to realize an interface between the microplate and the end effector of the slider in such a way that the plate is positioned by a technician always in the same starting position and it is also fixed during handling and reagents dispensing.

4.5 Robotic tips

Nowadays Inpeco makes use of robotic tips with a volume range too high for the cross-matching dispensation. For our intent, it was necessary to find a tip with an adequate volume range for the small quantities of interest and which, at the same time, guaranteed to dispense, with a single withdrawal, several reagents in several wells of the reaction plate.

In the first cross match phase technicians must dispense volumes from 5 to 15 microliter in each well. Consider having the maximum possible number of recipient sera, and therefore having to fill all 96 wells of the microplate.

The following table shows the volume per well to be dispensed and the maximum quantities (22 recipient sera) to be taken in each step of the first dispensing phase.

Step	Volume x well	Max volume x 22 sera
Donor cells dispensation	15 µl	1440 µl
Negative control dispensation	15 µl	60 µl
IgG positive control dispensation	15 µl	30 µl
IgM positive control dispensation	5 µl	10 µl
Serum dispensation	15 µl	60 µl

Table 9: First phase dispensation

The choice of the tip is one of the priorities for the experiments to give reliable results. For this first phase, we opted for the use of 1-50 microliter conductive tips from the Starlab group, which is specialized in the field of liquid handling technology and laboratory products.

Starlab robotic tips are engineered to the same specifications as the original equipment manufacturers' tips to ensure compatibility and a consistent fit. In this case, this tip is perfectly suitable to the Tecan devices from which the Inpeco robotic arm comes.

The 50 µl tip allows having up to three 15 µl dispensations x well with a single withdrawal of 45 µl. In this way, it is possible to fill all 96 wells of the plate with only 32 withdrawals, thus speeding up the manual procedure carried out today in the laboratory. Since the tip does not encounter the liquid already present in the well, it must only be changed with one equal when the reagent to be dispensed varies.

In the second dispensing phase the quantities to be dispensed are shown in the following table.

Step	Volume x well	Max volume x 22 sera
IgG fluorescent ab dispensation	40 µl	1920 µl
IgM fluorescent ab dispensation	40 µl	1920 µl

Table 10: Second phase dispensation

In this phase the volumes handled are decidedly greater and therefore this requires a change of the type of tip to be used.

The 40-400 µl Rainin robotic tip allows having up to ten 40 µl dispensations x well with a single withdrawal of 400 µl. In this way, it is possible to fill all 96 wells of the plate with only 5 withdrawals.

Both tips considered are equipped with the LTS system or Lite Touch system to improve the adhesion between the tips and the end-effector of the robot significantly. LTS reduces the force required to insert and eject the tips.

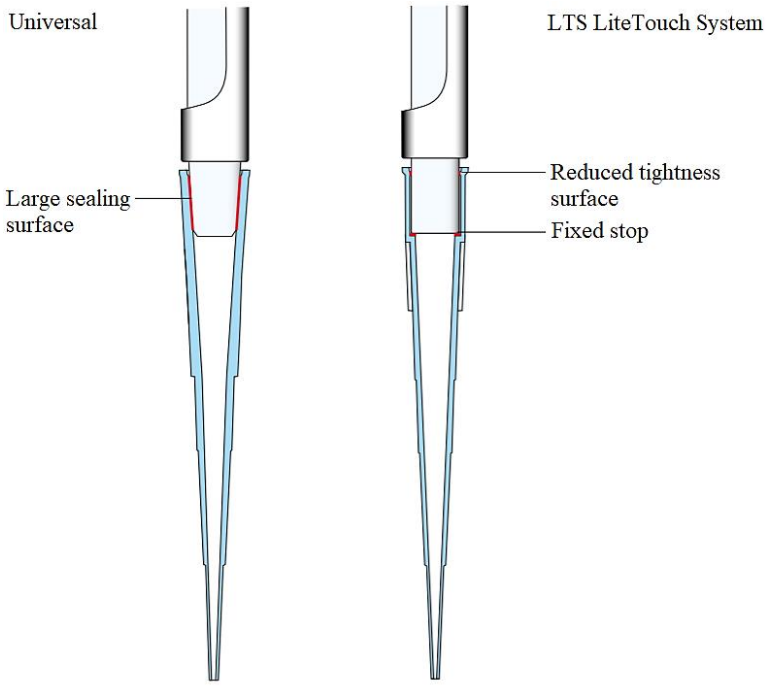


Figure 19: LTS system

5. Dispensing process

The pipetting process of the liquid handling system consists of several steps. Suppose to schematize a pipette above a tube filled with a biological liquid. The pipette represents the end-effector of the robotic arm with a tip at the end, while the tube represents the container with the reagent to be dispensed into the microplate wells.

During the first aspiration phase, the pipette is initially brought into a Z-start position and then is moved down substantially slower than the purely robotic movements in Y and Z.

During a second aspiration phase, an initial air gap is sucked from the pipette to create an interface between the system liquid and the biological sample to be collected. System fluid must never be contaminated with a biological fluid.

During a third suction phase, a capacitive liquid level detection circuit, which will be described in the following paragraphs, is activated and the pipette is slowly moved from the Z-start position in the direction of the liquid surface.

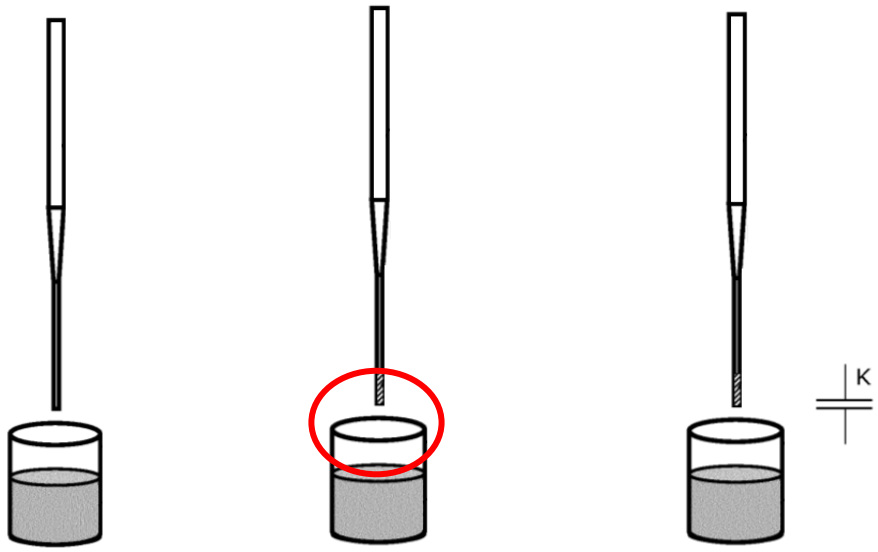


Figure 20: First three phases of aspiration

A fourth aspiration phase follows. The biological liquid is aspirated and suction takes place with LLD capacitive monitoring.

During the fifth aspiration step, the pipette is retracted for a clot step and the capacitive sensing checks plausibility based on the aspirated volume and tube geometry.

During the last aspiration phase, the pipette aspirates a transportation air gap to avoid dripping when moving towards the dispensing position and moves to a waste position. In this position, the pump dispenses all the content left in the tip into a waste tank and moves to another position where is discarded the tip.

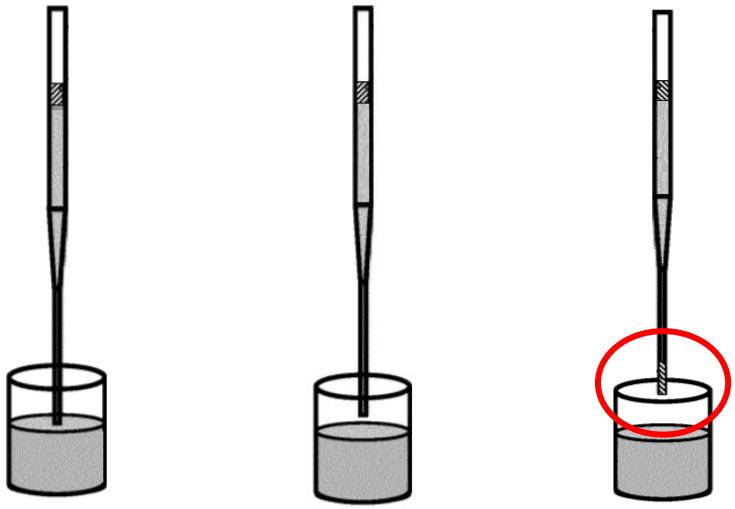


Figure 21: Last three phases of aspiration

Currently, in the Inpeco robotic arm, each withdrawal follows only one dispensing. Also, in the immunogenetic laboratory the technician, during the cross-match test, takes a single volume sample with a single dispensation for all the wells of the plate. For a manual procedure, this method reduces the human error probability and provide to easily control the correct volume to be dispensed.

In an automatic process, this could be avoided by following different dispensations after one withdrawal to speed up the process.

This is possible by taking the liquid quantity corresponding to the volume to be dispensed in a single well multiplied by the number of wells to be filled. Only at the end of the different dispensations, a transportation air gap is aspirated to avoid dripping during the stroke in the return to the next withdrawal or tip change position.

The process is illustrated in the figure below.

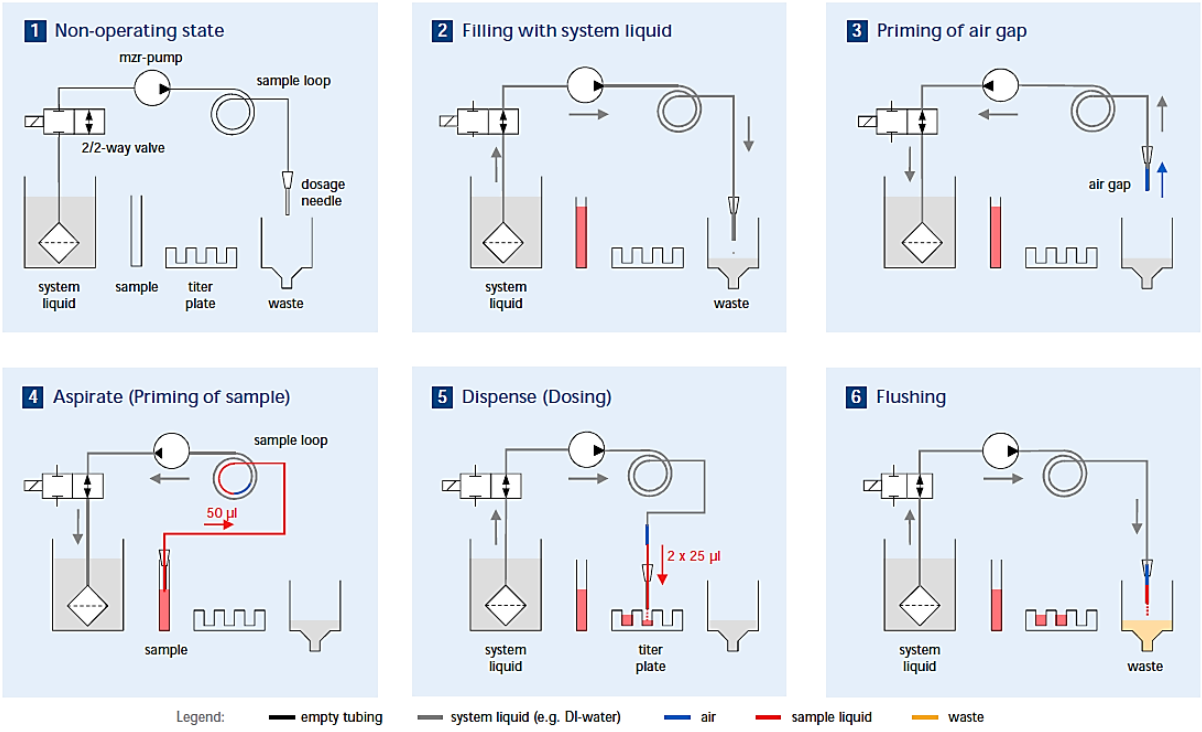


Figure 22: Dosing system

Before initiating the pump, the circuit must be filled with the system liquid through a specific valve. Later the pump aspires a predefined amount of initial air gap to avoid any contact between the sample and liquid system. The next step is to aspirate the biological sample desired corresponding to the total liquid to be dispensed in all plate well. The liquid is then distributed in the various wells and finally, the pump expels the remaining sample in an appropriate container.

6. Sensors

Inter-well contamination or other fluid handling errors during compatibility tests, such as the cross match between a donor and different recipient serum, can lead to false-positive or negative results that could endanger the health of the patient. For this reason, automatic liquid handling systems must be able to:

1. detect liquid levels to ensure that the pipette tip is inserted into the liquid at the correct depth;

2. detect the presence of clots to guarantee the aspiration and dispensing of the correct quantities of liquid without compromising the success of the test;

The Inpeco Aliquoter Module uses a capacitive method for both liquid and clot detection during the dispensing phase that can be integrated into the device of interest. Capacitive-based detection is based on the dynamic analysis of a capacitance signal monitored at tip level and works by sensing a variation of electric charge thanks to the difference of the dielectric constant between air and liquids.

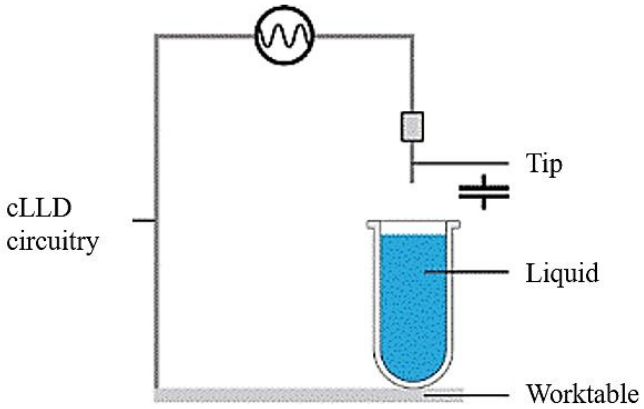


Figure 23: Capacitive detection circuitry

6.1 Liquid level detection

To ensure correct liquid level detection, the change in signal as the tip moves across the air-liquid boundary must be clear and rapid. In fact, as soon as the tip approaches the liquid surface, the signal increases and jumps when contact happens. In the same way, when the pipetting tip leaves the liquid, there is a drop capacitance signal.

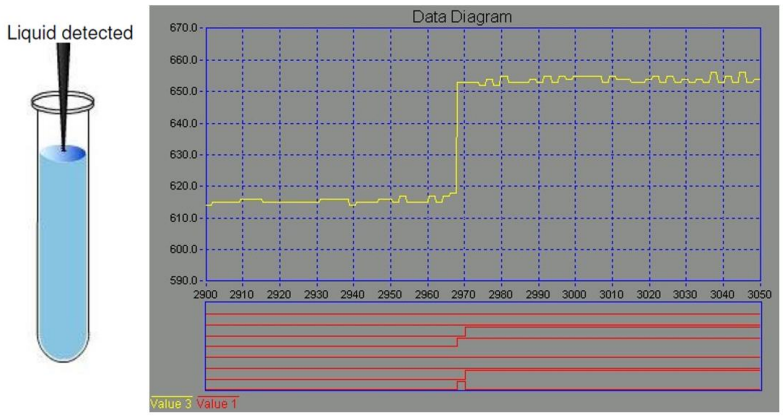


Figure 24: Tip/Liquid surface contact and capacitive signals

Tip goes different Z-step linked to the liquid/clot detection processes:

1. Z-Travel over the tube: Z-Motors works to aspirate a system air gap inside the pipetting channel to avoid contact between the sample and liquid system;

2. Z-Scan inside the tube: Z-Motors speed slow down at a predefined z-level and capacitive detection starts. It continues until the liquid is detected or the Z-Max level has been reached;

3. Liquid level detection: Z-Motors stops and a capacitive signal takes place;

4. Tip goes deeper with submerging steps: Z-Motor is set to “liquid level speed” given the tube geometry and expected aspirated flow;

5. Aspiration of the biological liquid: the pump is activated to withdraw the expected volume of liquid;

6. Retract step: the tip retracts slowly to a “clot step” verification level (explained in the next paragraph). It picks up a transportation air gap and it moves over the destination position.

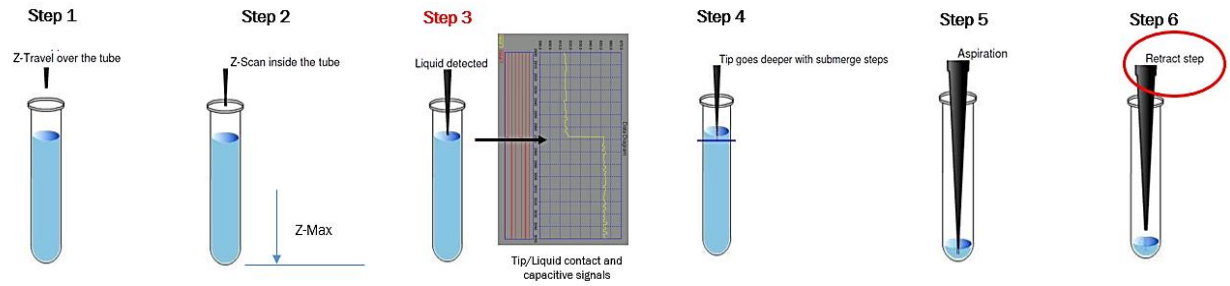


Figure 25: Liquid detection - High-Level Description

6.2 Clot detection

A clot consists of agglutinated fibers or particles. These particles can form a mass that is partially aspirated and hangs to the tip during the retracting step. During aspiration, if the tip is blocked by a clot, the volume remaining in the tip will be less than expected. This invalidates the entire test.

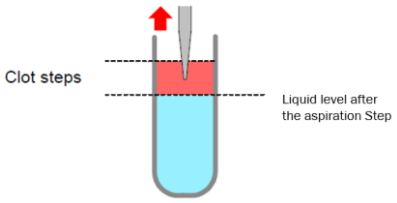


Figure 26: Clot detection - High-Level Description

Once the aspiration step is finished, the robotic arm goes up for the "clot step". If, in this new position, the capacitive sensing does not detect liquid around the tip, this means that the pump aspirated the expected volume without any occlusion, otherwise a clot error is generated.

The clot, therefore, indicates a plugged tip and that the aspirated volume is not the volume that is expected but is smaller. This volume goes from zero (clot on the surface) to a larger volume but always lower than desired.

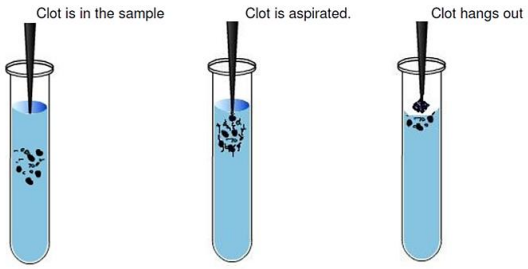


Figure 27: clots at different levels

7. Loading/unloading process tip

In the manual cross match procedure, the laboratory technicians, during the dispensing phase, take the amount of reagent corresponding to a single well after choosing the appropriate tip, deposit the liquid inside the desired well by immersing the tip, throw away the tip and repeat the procedure for all wells. In this case, the tip change at each well is absolutely necessary to avoid the risk of contamination that would compromise the entire test.

In the robotic procedure, this does not happen. The tip, during the dispensing phase, never meets the liquid and, for this reason, the tip change takes place only when the reagent to be dispensed changes. In the laboratory this method could also be used but, having to proceed manually, it is not possible to guarantee that the tip does not encounter the liquid due to the small quantities of liquid to be treated. A human error, in this case, could give rise to cross-contamination leading to an error in reading the results.

Hence, every time the pipettor arm needs to change the reagent to be dispensed, the tip must be replaced with a new one. This tip loading/unloading process takes place in the device shown below.

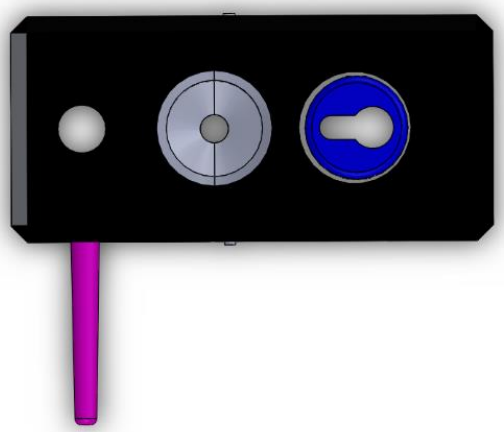


Figure 28: Tip Loader (top view)

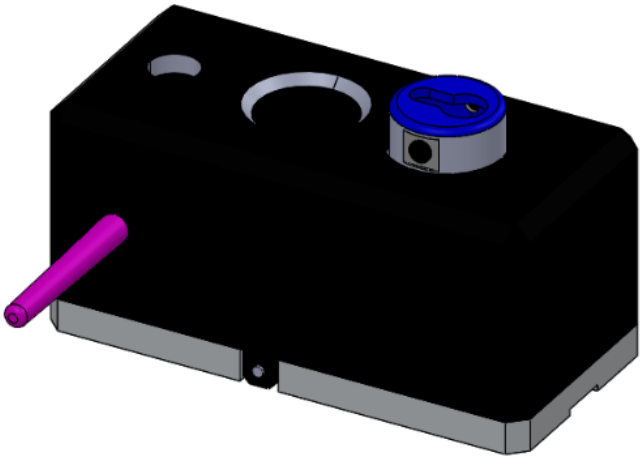


Figure 29: Tip Loader (side view)

After taking the biological sample from the Eppendorf tube and dispensing it into the appropriate wells of the reaction plate according to the plate scheme, the end-effector of the pipettor arm moves towards this block.

The first step is tip removal. The robot, once it reaches this position, fits into the first hole on the right in the previous figure. This hole in turn consists of a larger hole with a thinner slot. First, the end-effector moves vertically and fits into the largest hole, then moves horizontally along the slot and finally, moving upwards, as the slot is narrower, the tip fits by remaining adhered to the hole and falls into a basket.

In a second time, the end-effector, deprived of the previous tip, enters the second hole where it is washed and subsequently enters the third hole on the left in the figure where it is fitted to a new tip. The tips are placed by technicians in a box and a system takes them one by one horizontally and places them vertically so that the robot can fit into them in the third hole.

Since in the specific case it was decided to use a 50-microliter tip for the first phase and a 400-microliter tip for the second phase of the cross-match test, the technician must change tips between the two phases. For this reason, it is possible to replace the previous tips with the new ones or a two-way box could be created so that the tip loader can load the correct tip for both dispensing steps.

The final device will have two accesses:

- access to allow the operator to manually load the tips;
- access to allow the operator to manually empty the tips already used;

8. Total robot

8.1 3D model description

The assembly of all the elements presented up to now represented the last step of the project in question. All the components were accommodated on a designed surface as can be seen in the following figures.

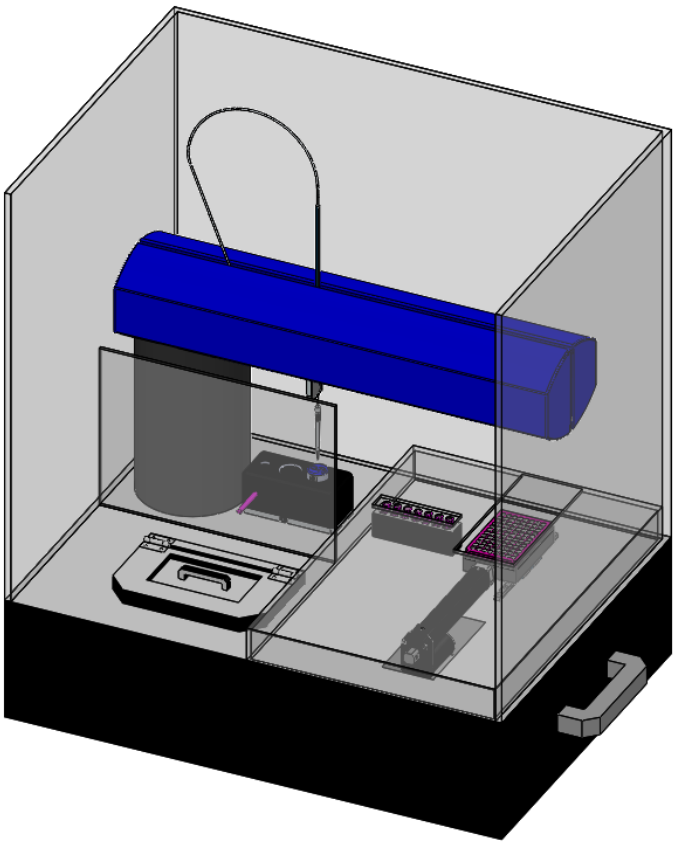


Figure 30: Robot 3D model (front)

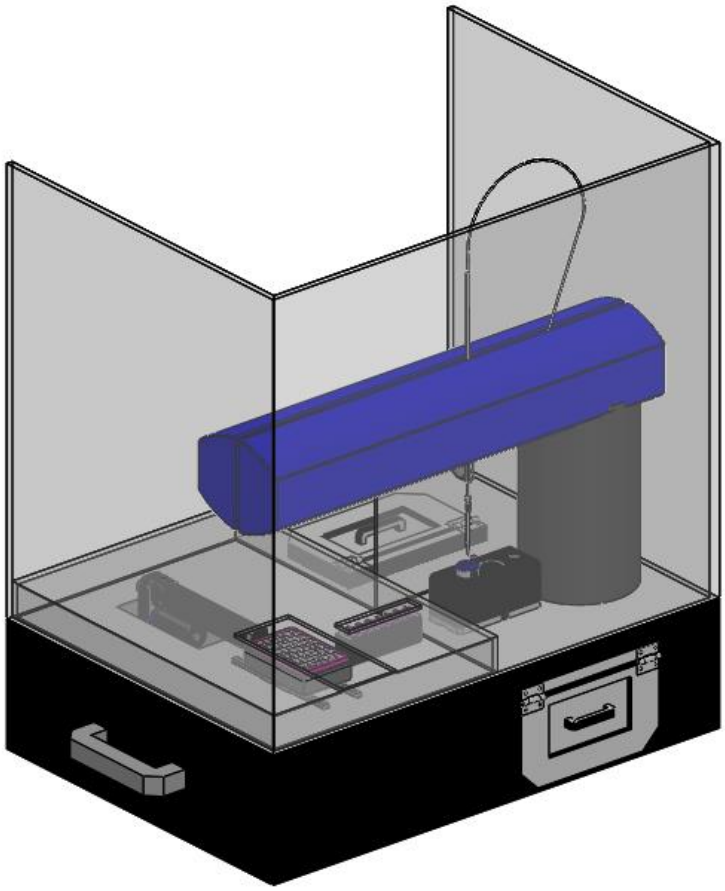


Figure 31: Robot 3D model (back)

On the surface it is possible to find:

- The one-channel 2-axis robotic arm inside which is inserted the microanular gear pump;
- The tip loader for the removal and loading of the tip;
- A part that represents the third degree of freedom of the system consisting of a linear actuator that moves the support for the microplate by sliding it on two profiled linear guides;
- Tube support for accommodating the Eppendorf tube with the reagents to be dispensed;
- Two tanks accessible to the operator: one in front of the pipettor arm in which to load the new tips and one behind the pipettor arm to unload the tips already used;

The robot will take reagents from Eppendorf tubes positioned by the operator in the appropriate support and will dispense them into the microplate wells according to the scheme plate.

The device must be constituted of all essential equipment and accessories to be used by the operator in total safety conditions according to the 2006/42/CE directive. According to this, protective devices must be designed and incorporated into the system so that it is not possible to start the moving parts if the operator can reach them and people cannot access mobile items in movement.

For this reason, several covers have been added to the system to ensure that human-machine interaction takes place in total safety. The microplate and Eppendorf tube supports and the tip loading and emptying tanks are the only elements that were made accessible to the operator.

Whether the mass, size or shape of the system or its various elements allow it to be moved by hand, the device must be easily movable and equipped with gripping devices that allow it handling safely. To evaluate this possibility of handling the system, an estimate of the total weight was made. According to Legislative Decree 81/08, the maximum limit of occasional weights possible to be handled is identified as 25 kg for workers, 15 kg for female workers. In the case in question, a weight of more than 25 kg was estimated as can be seen in the following table.

Component	**Weight (g)**
Robotic Arm + Tip Loader + Micropump	7000
Actuator	1500
Linear Guides	20x2
Plate support	20
Tube support	100
Cover	100
Support surface + 2 tanks	40000
TOT	**~ 50 kg**

Table 11: Components Weight

Therefore, it is not possible to manually move the entire device but it was equally equipped with handles to facilitate the transport with the help of other devices.

8.2 Construction project

The result is given by the assembly of elements coming from different manufacturers. The figure below shows the 3D exploded view of the device specifying the supplier for each element.

POS	MFR	Description
1	Inpeco SpA	Robotic Arm
2	Inpeco SpA	Tip Loader
3	HNP Mikrosysteme	Micropump mzr-2921X1
4	Ewellix	Linear Actuator
5	Ewellix	Linear Guides
6	TBD	Plate Support
7	Falcon	96-well Microplate
8	TBD	Tubes Support
9	Eppendorf	Tubes
10	TBD	Cover
11	TBD	Cover
12	TBD	Support Surface

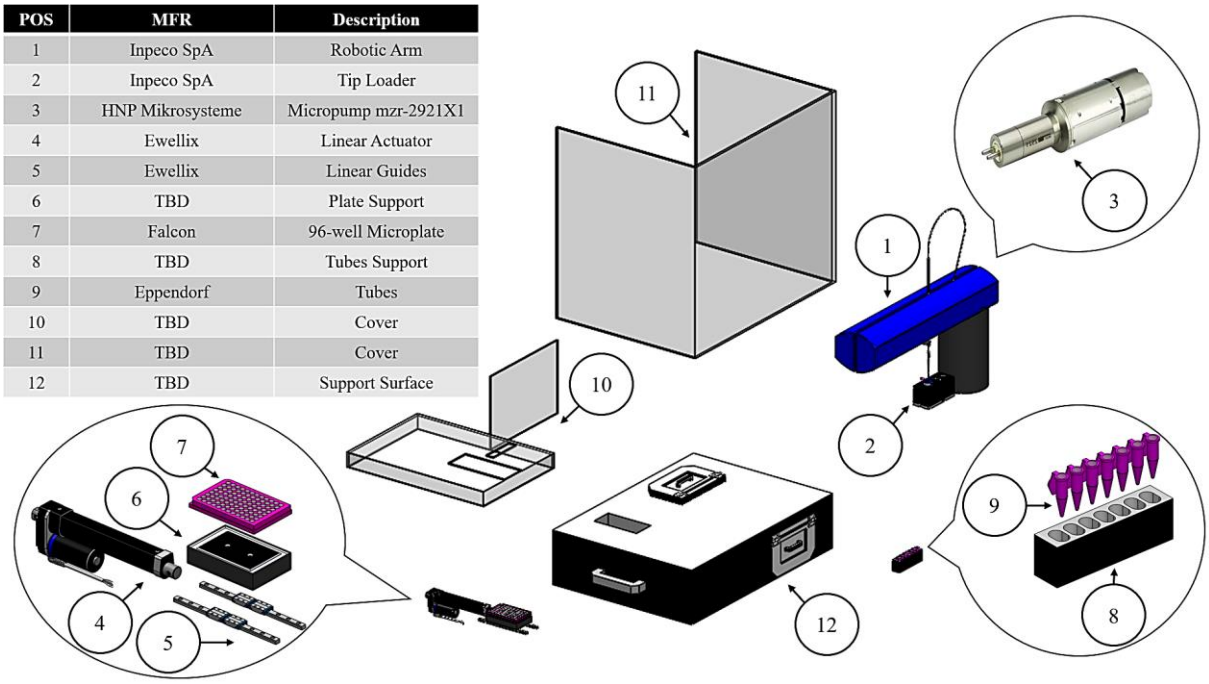


Figure 32: 3D exploded model

In the table shown in the figure, there are the identification numbers of the individual elements in the first column, the manufacturer and the description of the specific element respectively in the second and third columns. The covers, the supports for the plate and the Eppendorf tubes and the support surface are associated with a TBD manufacturer. The acronym TBD "to be defined" refers to elements that are easy to make and for this reason, it is not necessary to specify a manufacturer, but this figure can be identified later according to the best offer.

Different suppliers are needed to make the whole device. It is necessary at this point to find a general contractor not being able to be the laboratory itself to assemble all the components. A general contractor is a person or a legal company, which is identified to optimize all construction processes. In a supplier park, it is the one that takes care of collecting all the material, assembling it and delivering it.

The idea could be to ask the Inpeco group to represent this figure to make him fully responsible for the final product. In this way, the realization of the device becomes easier to manage because

officially there is a single manufacturer. This would lead to the addition of this new robot to the current Inpeco production.

8.3 Space analysis

The following images show the dimensions of the robot and its encumbrances. All the specific dimensions are expressed in cm.

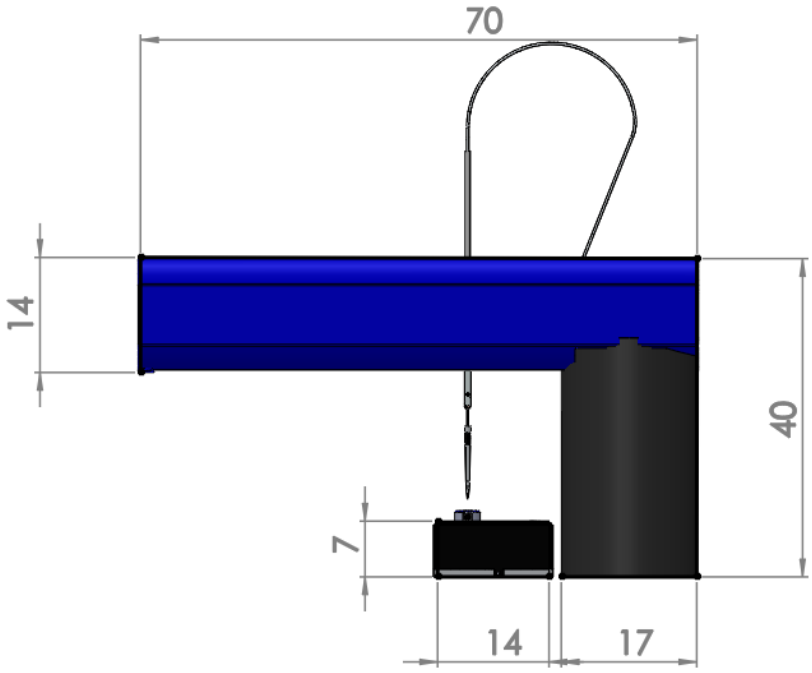


Figure 33: Robotic arm and tip loader dimensions

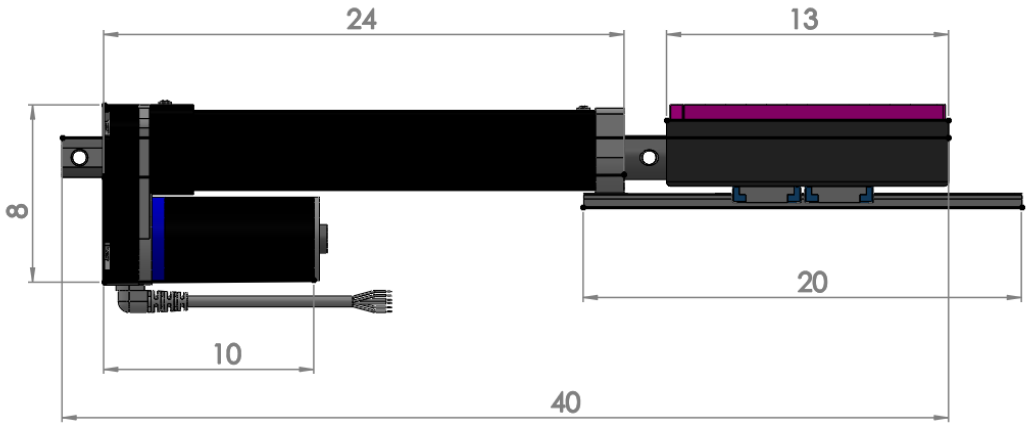


Figure 34: Slider dimensions (side)

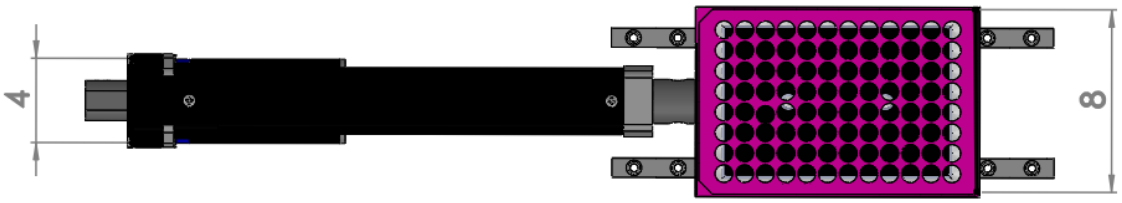


Figure 35: Slider dimensions (top)

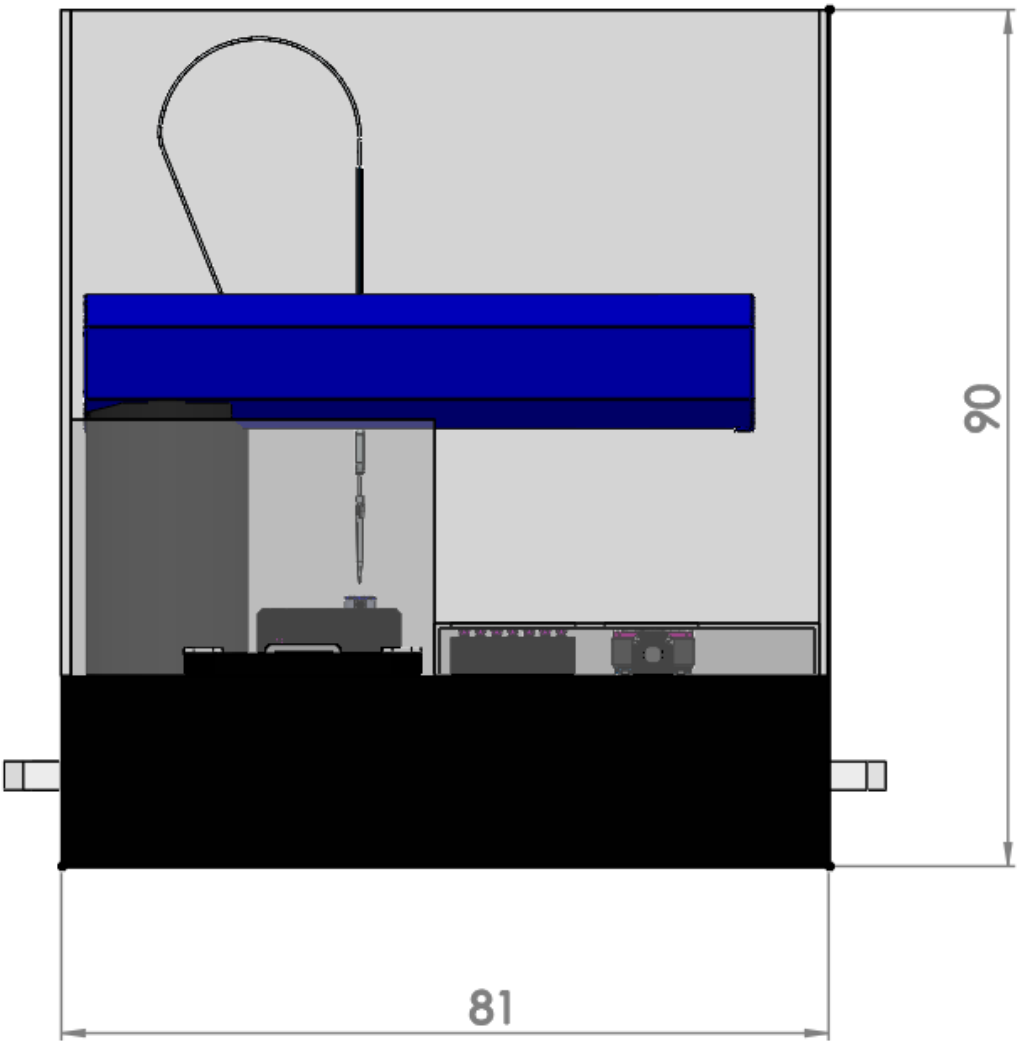


Figure 36: System dimensions (side)

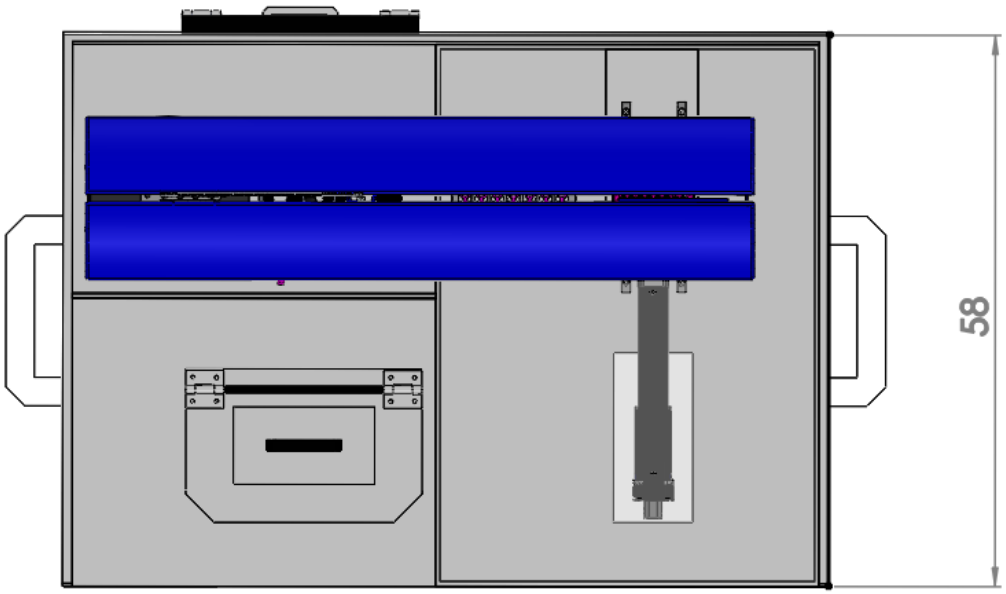


Figure 37: System dimensions (top)

8.4 Costs

No cost limits were fixed for the concrete realization of this project, however, every attempt was made to keep it contained. Some manufacturers provided a quote for the specific solution proposed while an estimate was made for the other components. The following table shows these results.

Product	**Cost (€)**
Inpeco Robotic Arm	5500,00
Inpeco Tip Loader	300
HNP Mikrosysteme Micropump	2000
Ewellix Actuator + Linear Guides	310
Covers + supports	500*
Support surface + 2 tanks	1000*
TOT	**~ 10000**

* estimated values

Table 12: Costs

The total cost of the product reaches € 10000.

8.5 Electronic and communication interface

After assembling all the elements, it is necessary to define a communication protocol that is the definition of the interaction modalities between the two-axis robotic arm with the pump and the linear slider to automate the cross-match test procedure. Both the pump and the actuator have integrated control, while in the robotic arm there is an electronic management board of the module itself in which the program is contained, thanks to which the robot carries out the movements. In the specific case, the board should be modified by inserting the program suitable for our application and considering the communication between robot and slider having added a degree of freedom to the system. Furthermore, the Inpeco module does not have any graphical interface through which the operator can interact with the device. Instead, it would be necessary to introduce a device (pc/touch monitor/app) in which to enter the minimum information enough for the complete and automatic execution of the test.

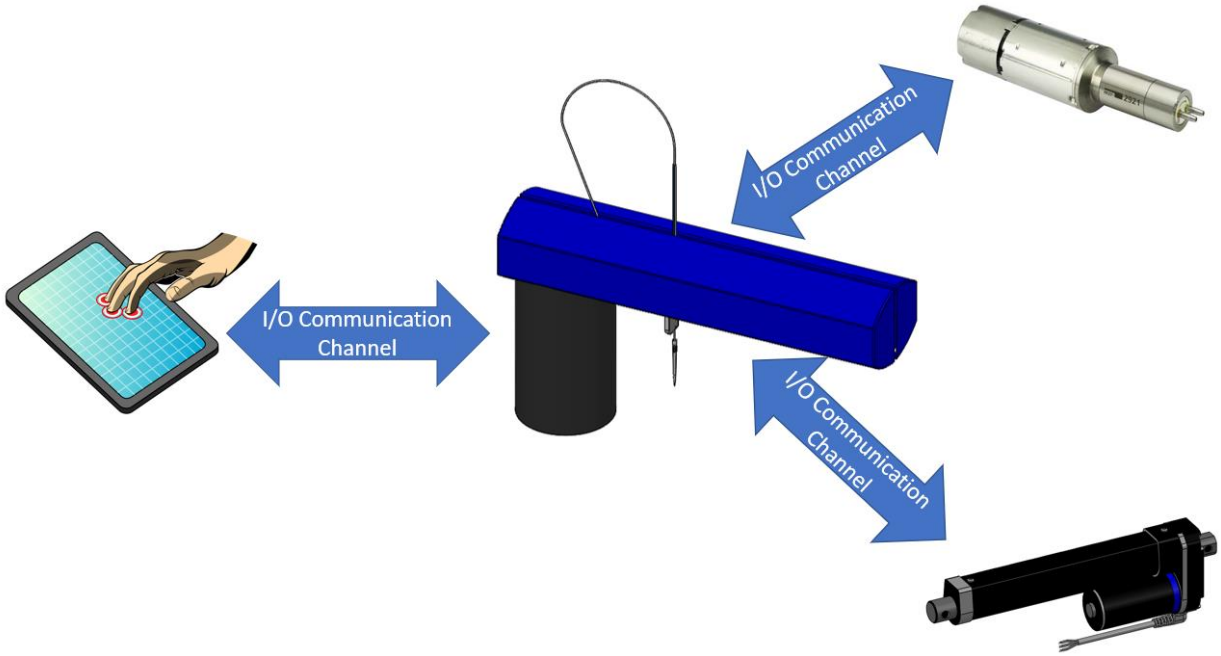


Figure 38: Communication interface

9. Execution of the algorithm

In this chapter, the execution of the dispensing device algorithm will be reported through the definition of flow charts. The cross match is carried out in two distinct phases between which the operator removes the plate for the incubation periods and washing steps. Before the device starts dispensing, the laboratory technician must perform 2 operations:

1. To prepare the Eppendorf tubes with the reagents for the first phase and the plate and insert them in the appropriate holder in the system

2. To enter the inputs necessary for the correct execution of the test through a graphic interface. These two inputs are:
    - Number of phase (1 or 2)
    - Number of available recipient sera

At this point, the automated cross match test can take place. The flow charts described below will refer to the maximum possible number of sera, but in the real case, some of them change according to the number of sera. Throughout the first phase of the cross match, the 50-microliter tip is used and is replaced at each reagent change, while in the second phase the 400-microliter one. In the flow charts that will be reported, the capacitive monitoring takes place in the "x microliter collection" block.

9.1 Donor cells dispensation

The first step that the device performs is the dispensing of the donor cells. Each well must contain 15 microliters of donor cells. The number of wells to be filled depends on the number of available sera according to the following formula:

$$n = 2\,(4 + 2\,x)$$

where n is the number of wells to be filled and x represents the number of sera available for testing. In this case, the flow chart is referred to the maximum number of sera.

The wells are filled to 2 by 2, hence with a 30 microliters withdrawal, 2 wells can be filled with 15 microliters each. Since the plate is made up of 8 rows, each column is therefore characterized by 4 withdrawals (circled in the following figure) and 8 dispensations. After dispensing in an entire column, the plate is then moved to allow dispensing the next column.

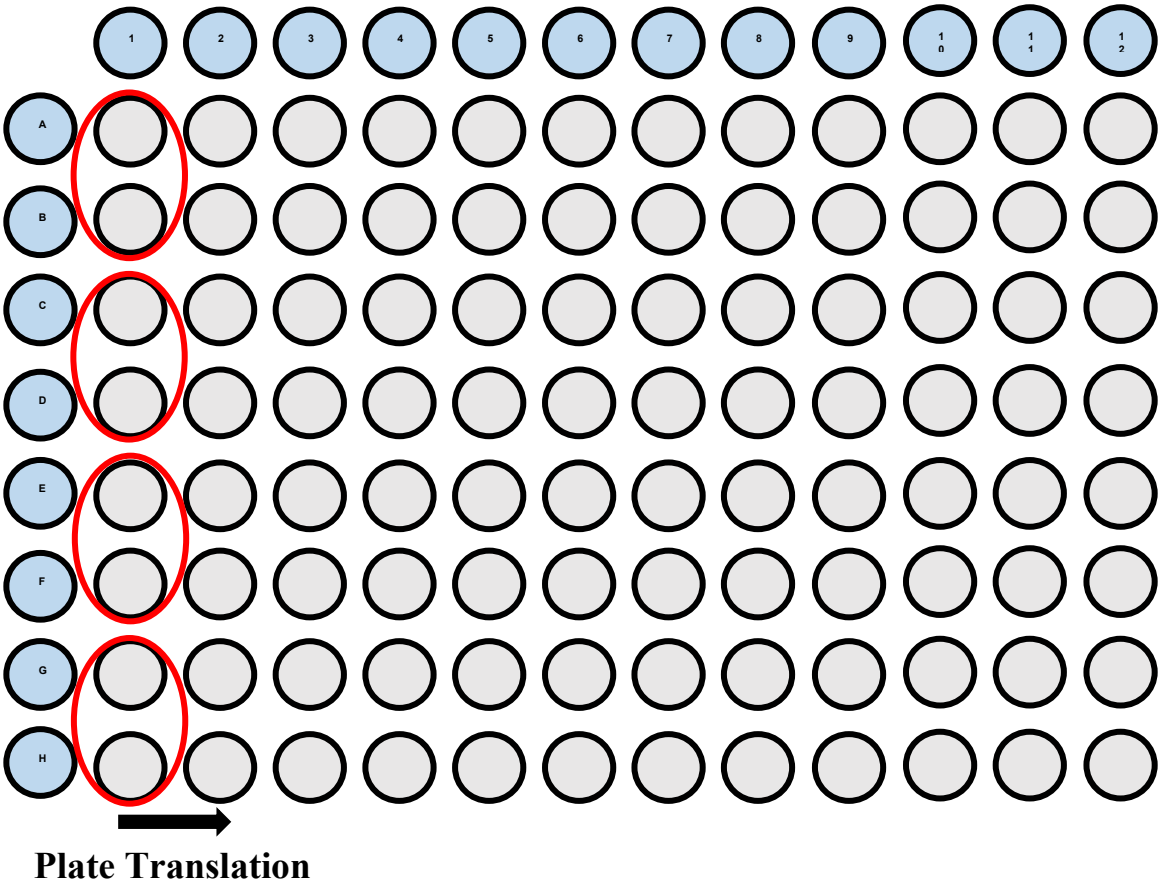


Figure 39: Donor cells dispensation

In the following flow chart, there are two different counters:

1. Column counter: counts the number of columns and goes from 1 to 13;
2. Withdrawal counter: counts the number of withdrawals per column and goes from 1 to 5;

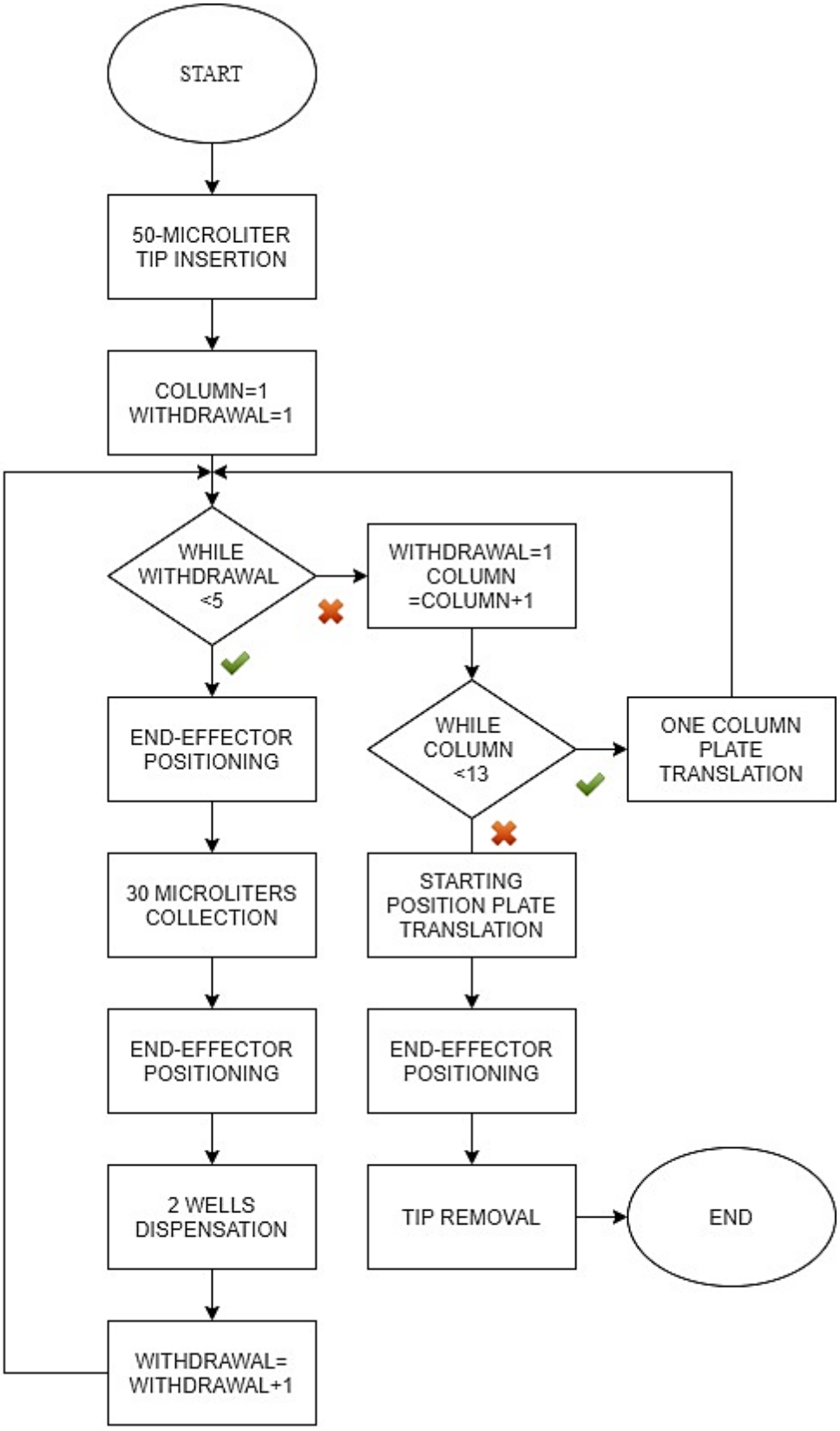


Figure 40: Donor cells dispensation flow chart

9.2 Negative control dispensation

The second step concerns the dispensing of negative control. Regardless of the number of sera, A1, B1, A7 and B7 wells must contain negative control 15 microliters each.

Also, in this case, the wells are filled to 2 by 2, hence with a 30 microliters withdrawal, 2 wells can be filled with 15 microliters each. This time each column is characterized by 1 withdrawal (circled in the following figure) and 2 dispensations. After dispensing in the first column, the plate is then moved to allow dispensing to the seventh column.

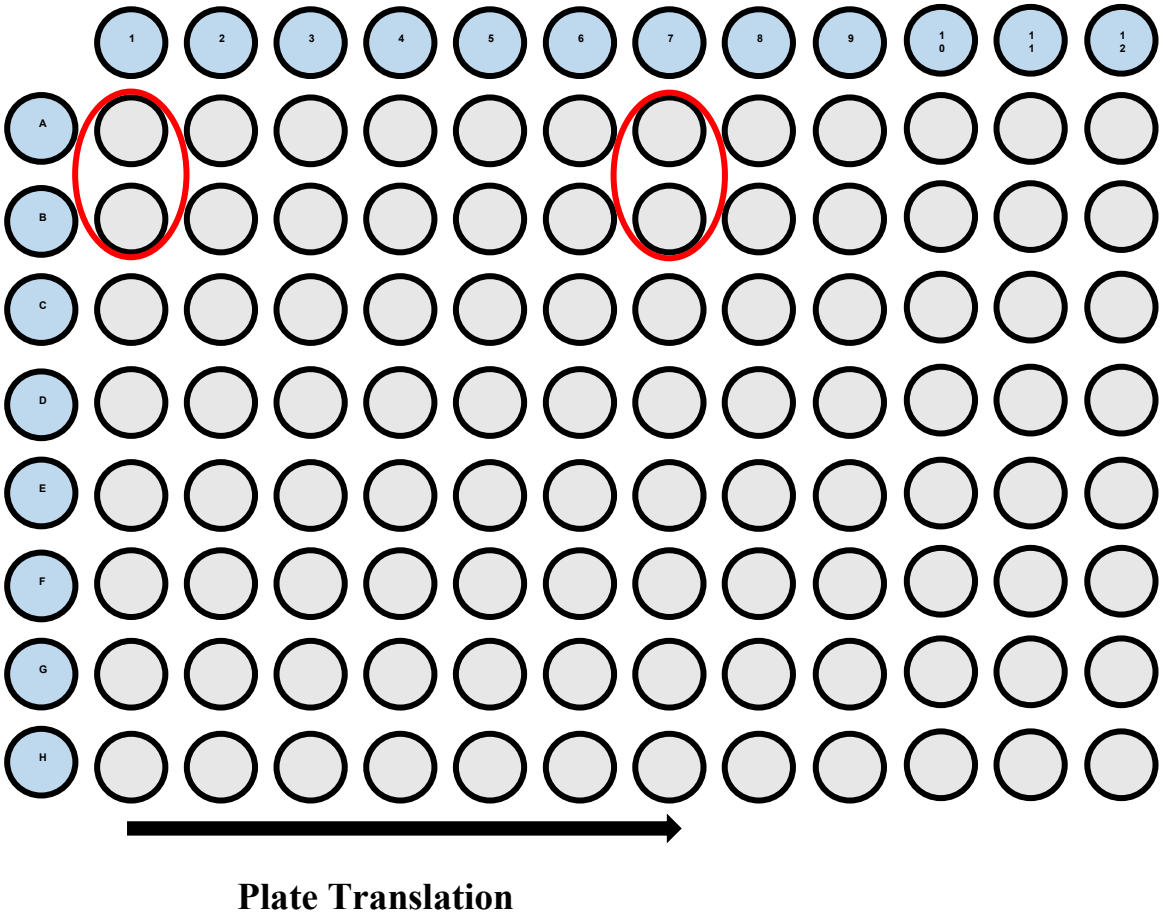


Figure 41: Negative control dispensation

In the following flow chart, there are two different counters:

1. Column counter: counts the number of columns and goes from 1 to 13;
2. Withdrawal counter: counts the number of withdrawals per column and goes from 1 to 2;

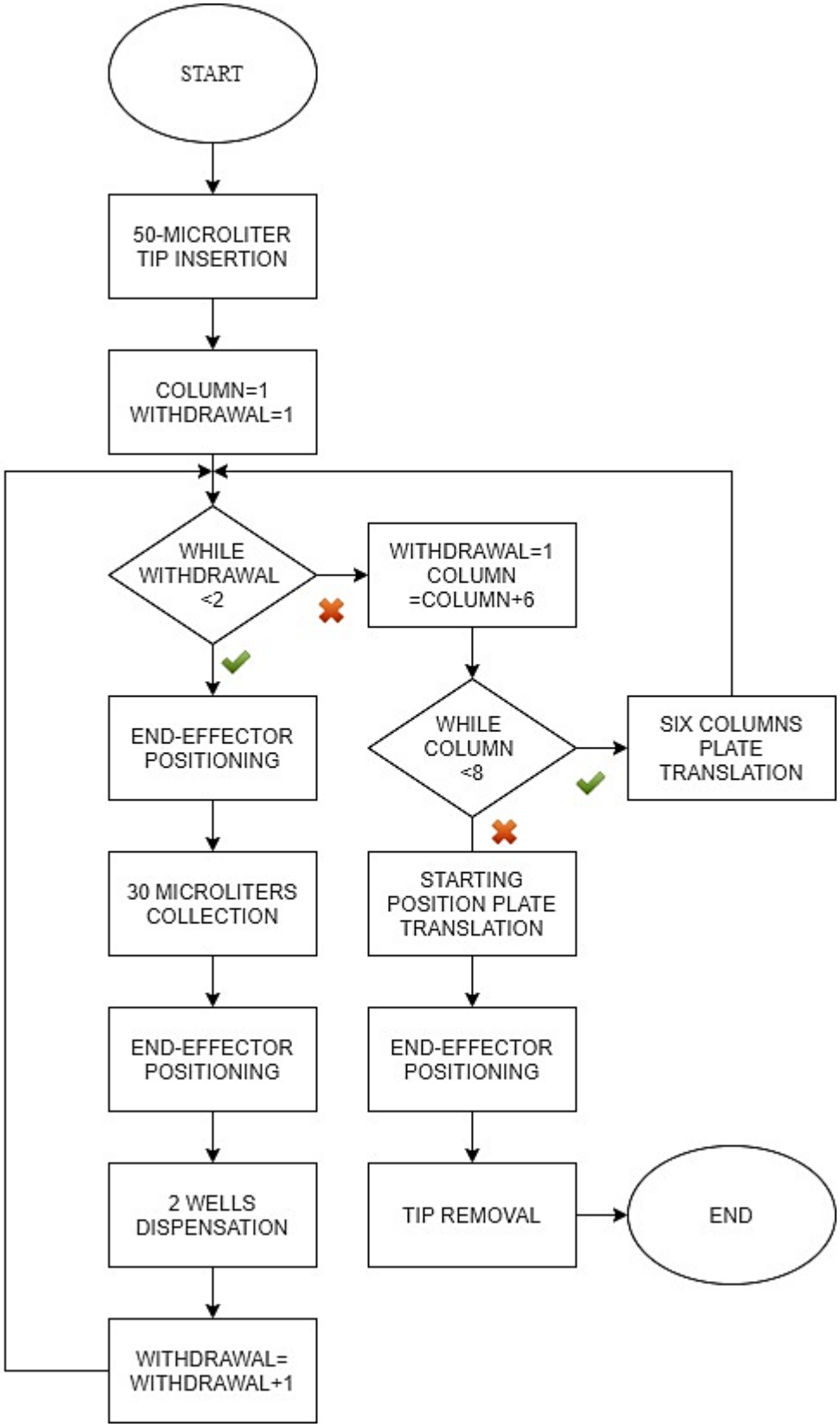


Figure 42: Negative control dispensation flow chart

9.3 IgG positive control dispensation

The third step concerns the dispensing of the IgG positive control. Regardless of the number of sera, C1 and D1 wells must contain IgG positive control 15 microliters each.

Also, in this case, the wells are filled to 2 by 2, hence with a 30 microliters withdrawal, 2 wells can be filled with 15 microliters each. This time the 2 wells to be dispensed are in the first column, hence the plate slider does not intervene.

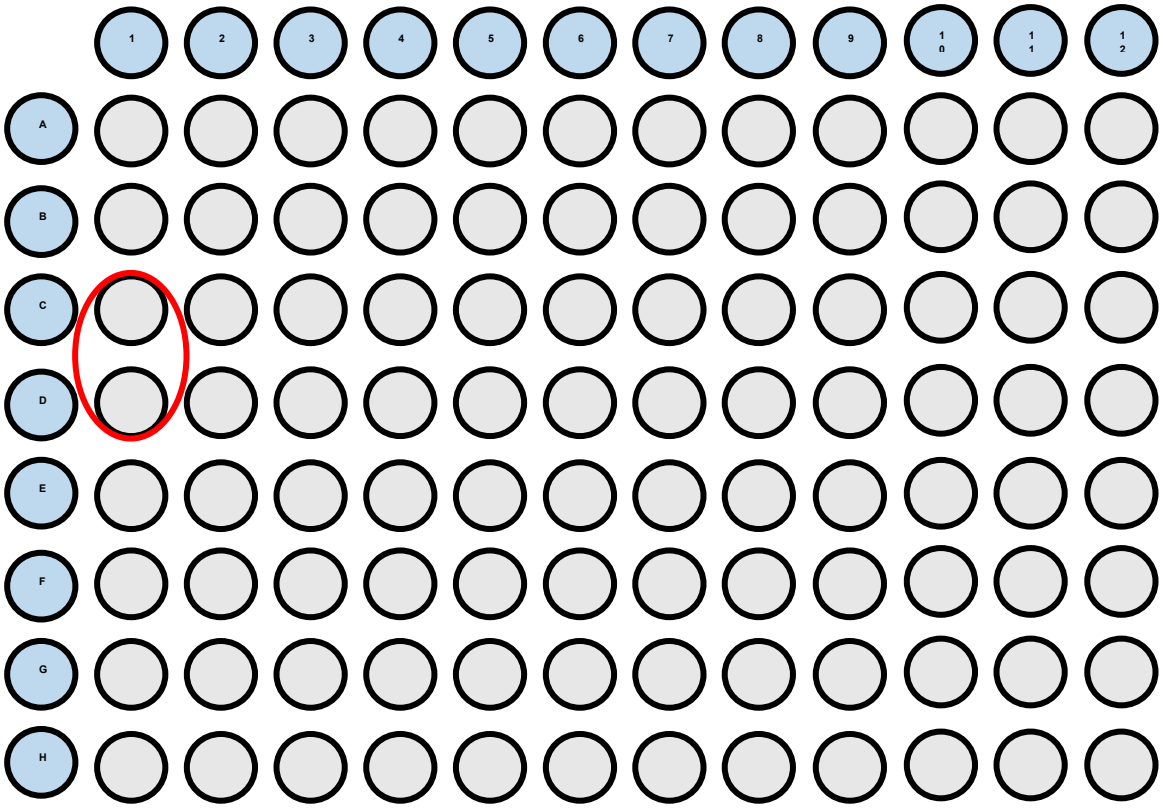


Figure 43: IgG positive control dispensation

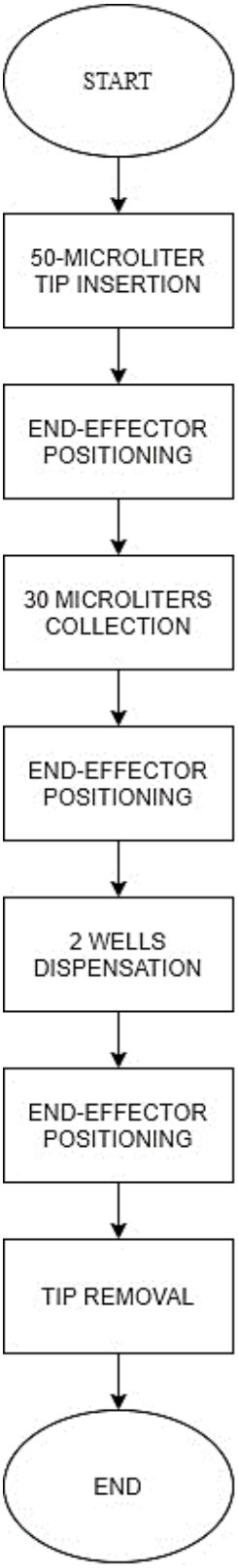


Figure 44: IgG positive control dispensation flow chart

9.4 IgM positive control dispensation

The fourth step concerns the dispensing of the IgM positive control. Regardless of the number of sera, C7 and D7 wells must contain IgM positive control 5 microliters each.

Like the previous case, with only one collection it is possible to fill both wells. With a 10 microliters withdrawal, 2 wells can be filled with 5 microliters each. This time, the specific 2 wells are in the seventh column, hence, before starting dispensing, the plate slider must move until the correct column. Once dispensing is finished, the slider must return to its initial position.

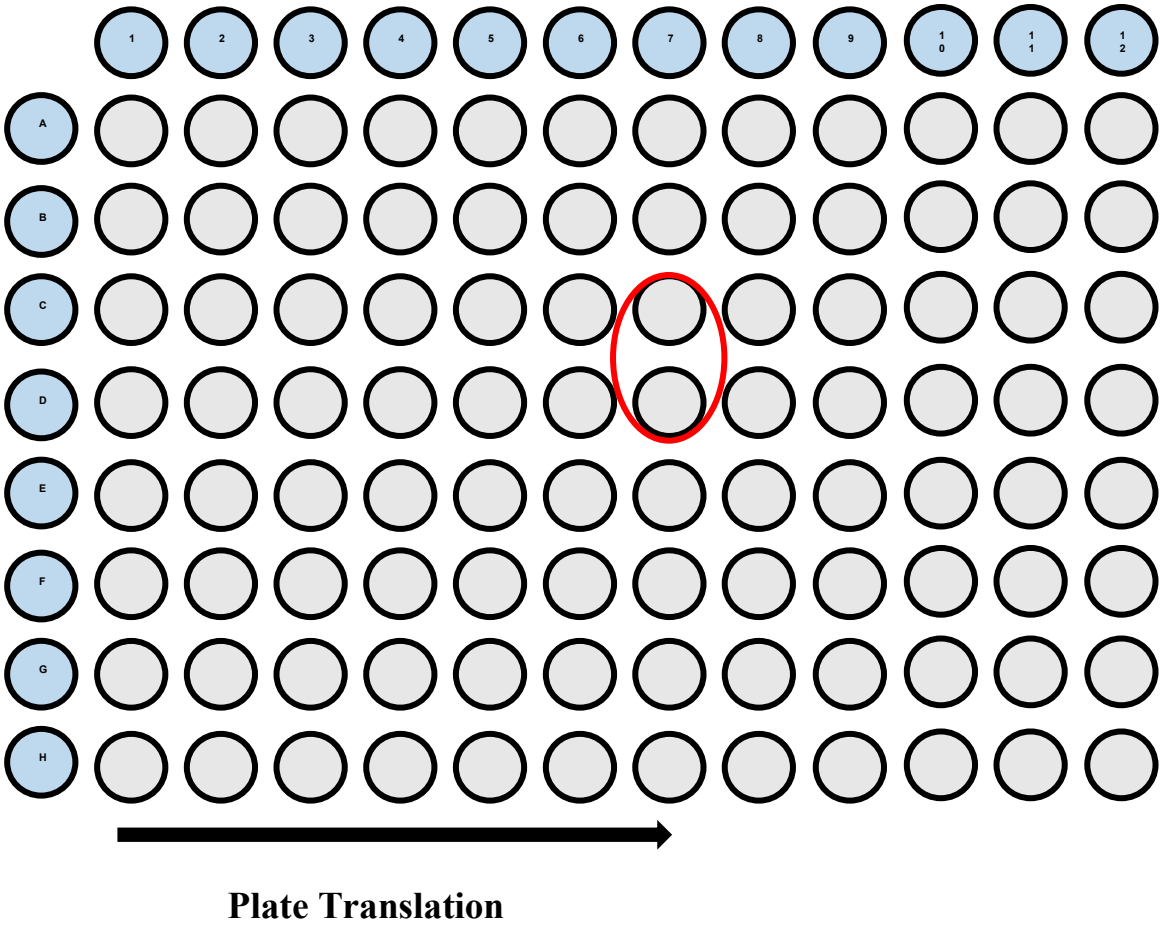


Figure 45: IgM positive control dispensation

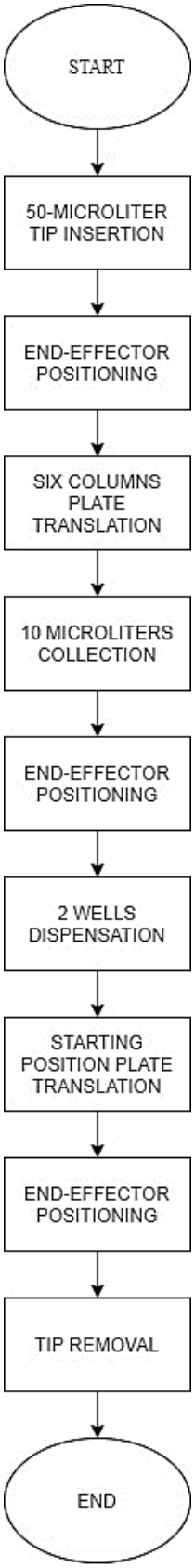


Figure 46: IgM positive control dispensation flow chart

9.5 Recipient sera dispensation

The fifth step concerns the dispensing of the different available sera. Regardless of the serum dispensed, the robot must always dispense in 4 wells with serum 15 microliters each, but the wells to be filled change according to the serum dispensed. Once the robot finishes dispensing a serum, before proceeding with the next serum, it must change the tip and replace it with a new one.

The wells are filled again to 2 by 2, hence with a 30 microliters withdrawal, 2 wells can be filled with 15 microliters each. Each column is characterized by 1 withdrawal and 2 dispensations. After dispensing in a column, the plate is then shifted six columns right. Once dispensing is finished, the slider must return to its initial position.

The following figure shows the wells to be filled for each serum (1-22).

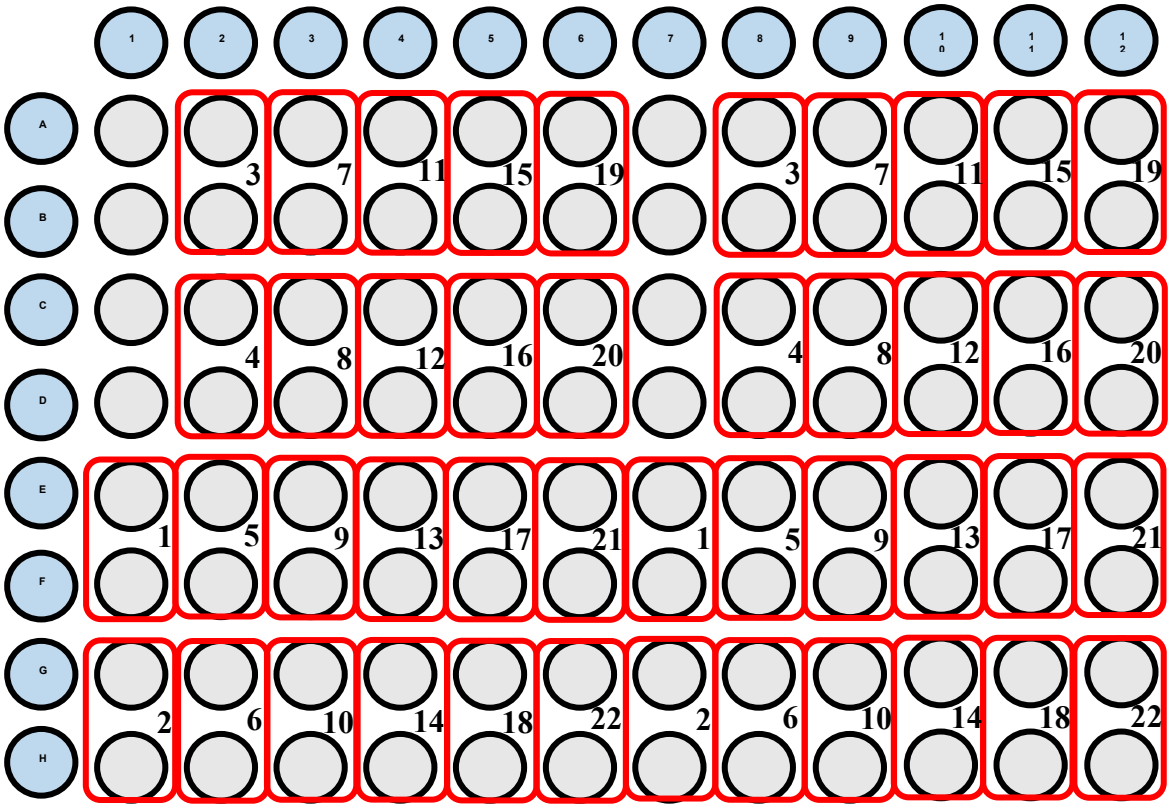


Figure 47: Sera dispensation

In the following flow chart, there are two different counters:

1. Column counter: counts the number of columns and goes from 1 to 13;
2. Withdrawal counter: counts the number of withdrawals per column and goes from 1 to 2;

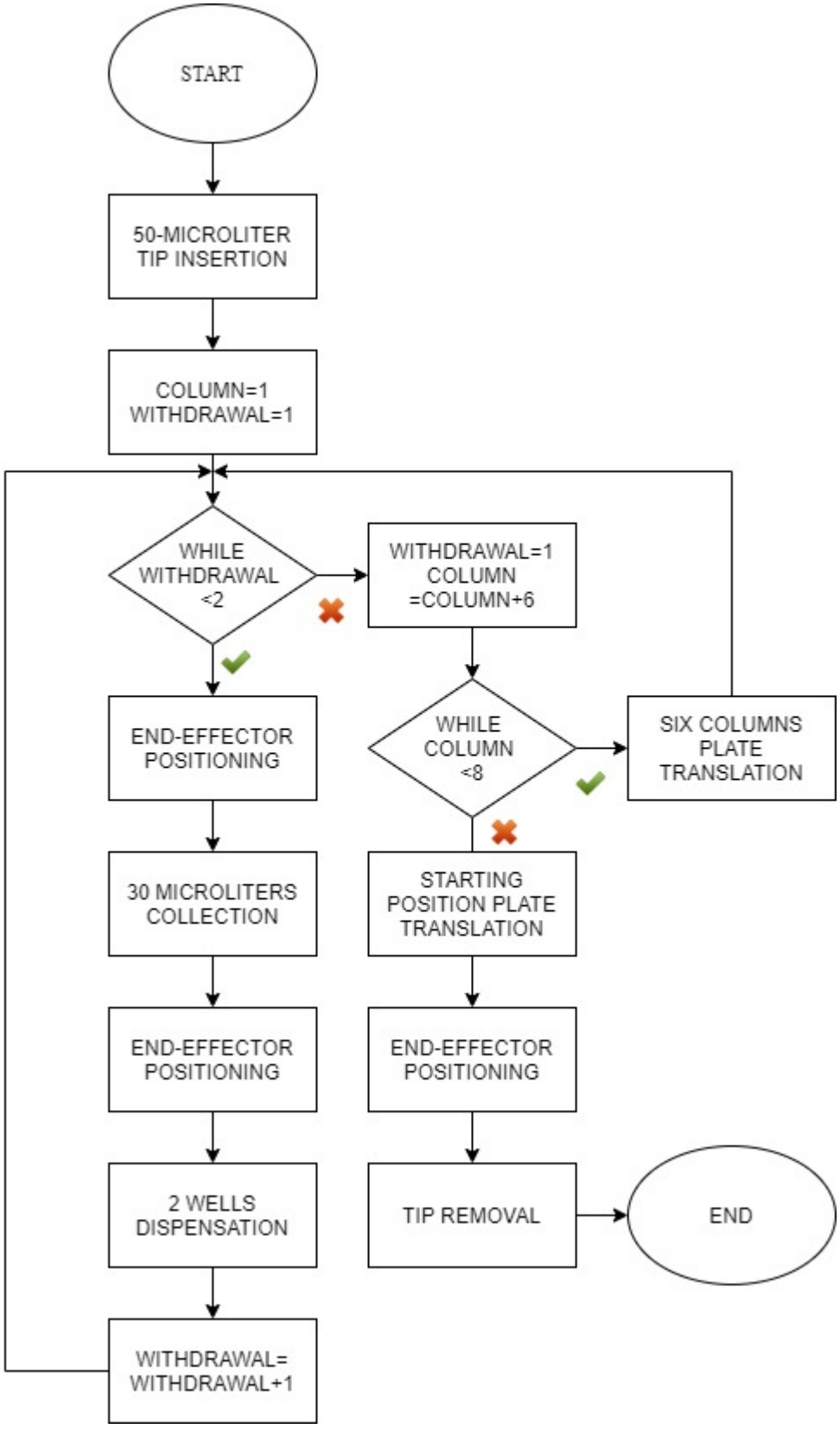


Figure 48: Serum dispensation flow chart

9.6 Phase 1 - Manual operations

Once the previous phase is completed, the lab technician removes the Eppendorf tubes with the remaining reagents and the microplate from the corresponding holders. During this period, the operator carries out the following operations:

- 20 min/22 °C incubation period;
- 2 washes in PBS solution: In this part, the technician uses a multichannel pipette (8 channels) to pick up and release PBS solution in all the plate's wells. Then there is the plate's centrifugation (1600 rpm/1min) and, at the end of the first wash, the technician manually empties the plate by turning it over and then dabbing it with paper for removing any excess. This procedure is repeated another time for the second wash;
- Preparation of the 2 new Eppendorf tubes useful for the second phase of the cross match;
- Tank emptying of the tips used and replacement with 400-microliter tips;

After the manual operations, the operator places the new reagents and the plates in their respective supports in the system.

9.7 IgG solution dispensation

The first step of the second phase that the device performs is the dispensing of IgG solution. Each well of the left half of the plate must contain 40 microliters of this solution. The number of wells to be filled depends on the number of available sera according to the following formula:

$$n = 4 + 2\ (x)$$

where n is the number of wells to be filled and x represents the number of sera available for testing. The flow charts described below refer to the maximum possible number of sera. This time with a 320 microliters withdrawal, 8 wells can be filled with 40 microliters each. Since the plate is made up of 8 rows, each column is therefore characterized by 1 withdrawal (circled in the following figure) and 8 dispensations. After dispensing in an entire column, the plate is then moved to allow dispensing the next column.

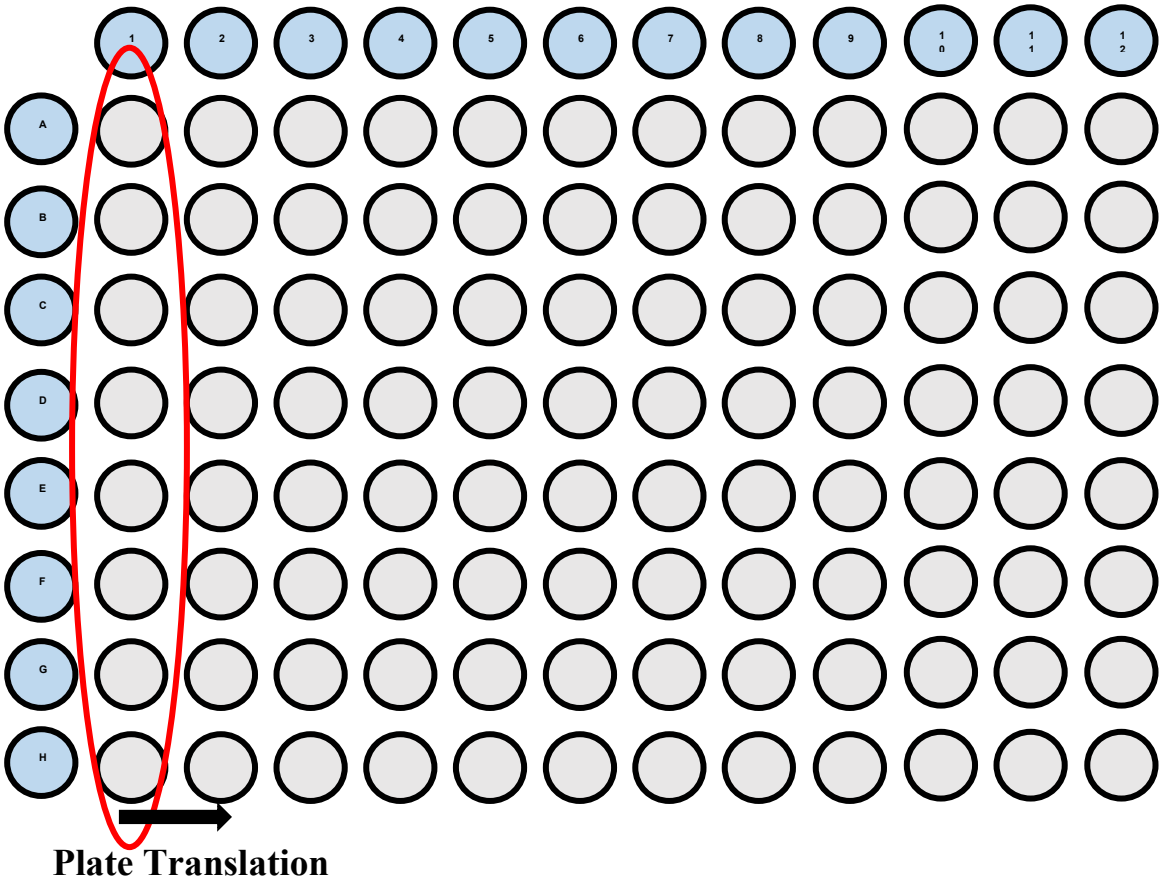


Figure 49: IgG solution dispensation

In the following flow chart, there are two different counters:

1. Column counter: counts the number of columns and goes from 1 to 7;
2. Withdrawal counter: counts the number of withdrawals per column and goes from 1 to 2;

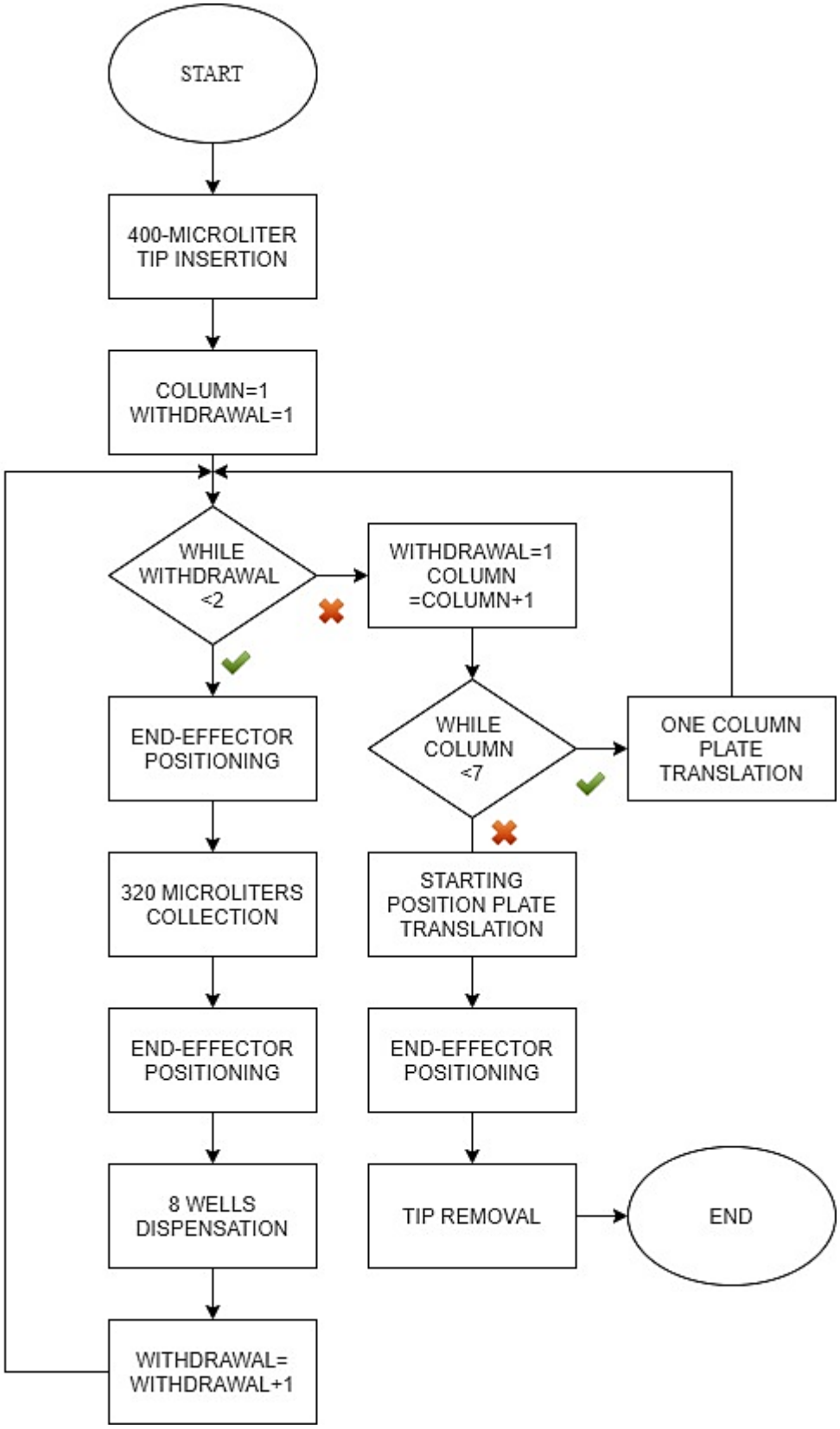


Figure 50: IgG solution dispensation flow chart

9.8 IgM solution dispensation

The second step of the second phase that the device performs is the dispensing of IgM solution. Each well of the right half of the plate must contain 40 microliters of this solution. The number of wells to be filled depends again on the number of available sera according to the previous formula. The flow charts described below refer to the maximum possible number of sera. Also, this time with a 320 microliters withdrawal, 8 wells can be filled with 40 microliters each. Since the plate is made up of 8 rows, each column is therefore characterized by 1 withdrawal (circled in the following figure) and 8 dispensations. After dispensing in an entire column, the plate is then moved to allow dispensing to the next column. This time, before starting the dispensing, it is necessary to translate the plate in correspondence with the seventh column and, once this step is completed and all columns are dispensed, the plate must return to the starting position.

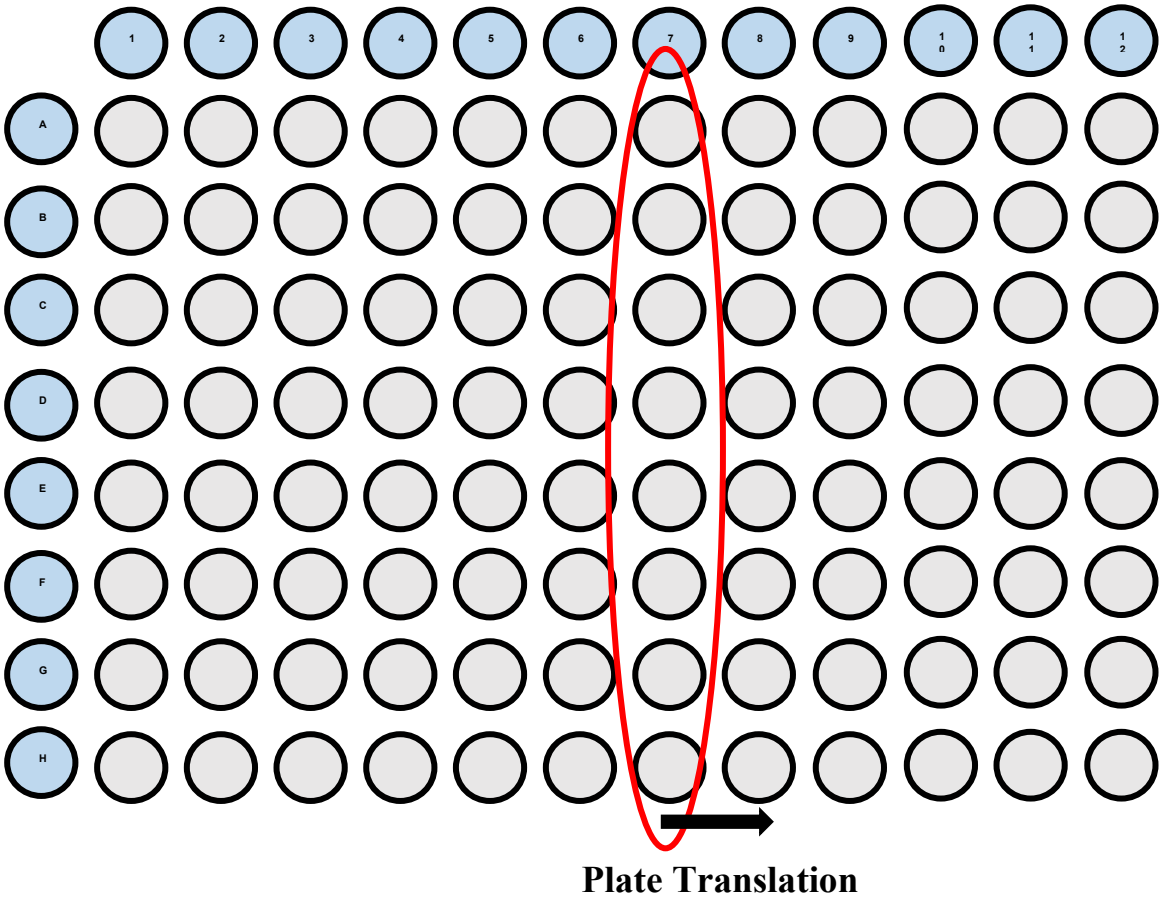


Figure 51: IgM solution dispensation

In the following flow chart, there are two different counters:

1. Column counter: counts the number of columns and goes from 1 to 7;
2. Withdrawal counter: counts the number of withdrawals per column and goes from 1 to 2;

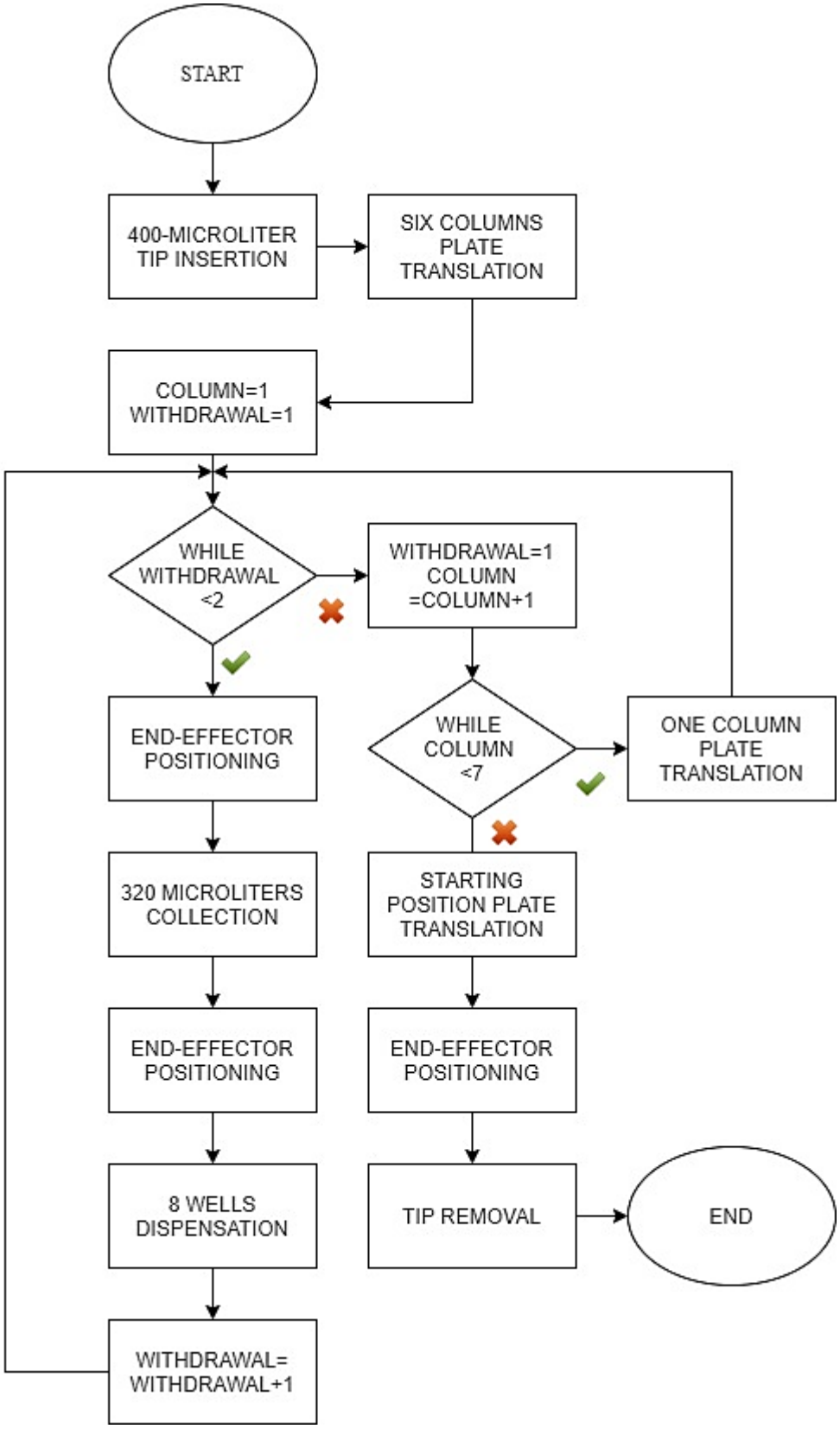


Figure 52: IgM solution dispensation flow chart

9.9 Phase 2 - Manual operation

Once phase two is completed, the lab technician removes the Eppendorf tubes with the remaining reagents and the microplate from the corresponding holders. During this period, the operator carries out the following operations:

- 10 min/22 °C incubation period;
- 1 wash in PBS solution;
- Tank emptying of the tips used and replacement with 50-microliter tips;

At this point, the cross-match test can be considered finished and, in the next step, this test is evaluated through a flow cytometer to verify compatibility between donor and recipient patient.

10. Conclusions

In this thesis work, a biological liquid handling system was developed to create a device that faithfully replicates the cross-match test, now performed manually in the Immunogenetics laboratory of the Molinette hospital in Turin. The manual procedure implies a high risk of error and, for this reason, the co-presence of two people is required at different stages of the test. Any error could compromise the success of the test and make the result unreliable. The most critical phase of the cross-match test concerns the dispensing of the reaction reagents at the microliters level into the wells of a microplate according to a specific scheme. This project focused on this phase by designing an automatic dispenser capable of handling these samples quantities with very high precision, drastically reducing the probability of error.

The first phase of the work involved the in-depth study of the biological aspects at the base of the test and the analysis of each step of the procedure at the laboratory collecting the problematic and most critical aspects. Having achieved a clear idea of the procedure to be automated, we looked for a company specialized in the automation sector in the biomedical field that could collaborate. Inpeco SpA was interested in supporting the realization of this device. The work started with Inpeco's Aliquoter Module. This robot is designed for a different application by dispensing biological liquids from a mother tube to daughter tubes through a 2-axis robotic arm. This module is unusable for the automation of the cross match test because it treats much larger quantities of samples not guaranteeing adequate precision and accuracy for the volumes of our interest and ha only two degrees of freedom that prevent dispensing in a reaction plate with xy-matrix. To make this device usable, it was first necessary to change the micropump inside the arm to reach levels of accuracy at least equal to those of the devices used today in the laboratory. Lab technicians currently use mechanical air-displacement Eppendorf pipettes to dispense desired volumes with 97% accuracy. The idea was to have a similar or higher percentage than this. For this phase, the intervention of another specialized company was necessary to propose a micropump with these characteristics.

HNP Mikrosysteme develops and produced micro annular gear pumps to reach highly precise metering volumes in the microliter range and the smallest volumetric flows. Thanks to their indispensable technical support, HNP operators chose for this application mzr-2921X1 pump of the low-pressure pump series. Thanks to the high dosage precision, the HNPM pump can achieve higher accuracies than the Eppendorf mechanical pipettes manually used in the laboratory today.

The next phase involved solving the problem related to the two degrees of freedom of the system. The Inpeco robotic arm can only travel in the z and y-direction. For this application, it was necessary to add a third movement in the x-direction. The least expensive and easiest option to make proved to be to entrust the third dof to the reaction plate. In this step, several companies were considered to evaluate the best offer in the current market. Ewellix and Newport are qualified to be two of linear motion's leading suppliers. Our need was to have a linear guide that would allow a minimum stroke of 100 mm with a resolution equal to the distance between one well and another of the microplate. Ewellix proposed two different solutions. Both include two profiled linear guides above which the reaction microplate can slide, but in the first case, the motion is guaranteed by a ball screw rotated by a step motor while in the second case by a linear actuator. Newport instead proposed a complete motorized solution with the desired characteristics with the only disadvantage of being much more expensive than the two previous proposals. For this thesis work, the cheapest solution with the Ewellix linear actuator.

To accomplish the dispensation, it is necessary to use tips to be fitted to the end-effector of the automatic manipulator. The Inpeco Aliquoter uses Rainin 1100-microliter conductive tips specific for its application. For the automation of the cross match test, it was decided to use two types of tips, 50-microliter tips for the first phase of the procedure and 400-microliter tips for the second one, also to have several dispensations with a single withdrawal unlike what happens in the laboratory today.

The last step of the thesis work involved the assembly of the various components in a virtual 3D model, also introducing protection devices according to safety requirements imposed by the directives. Subsequently, the weight of the system, the analysis of the spaces and dimensions of the device were evaluated and an estimate of the cost that reaches the entire robot was proposed, achieving the objective that we had set ourselves: to propose a concrete and feasible solution with the characteristics of the new robot and a first estimate of the corresponding cost.

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