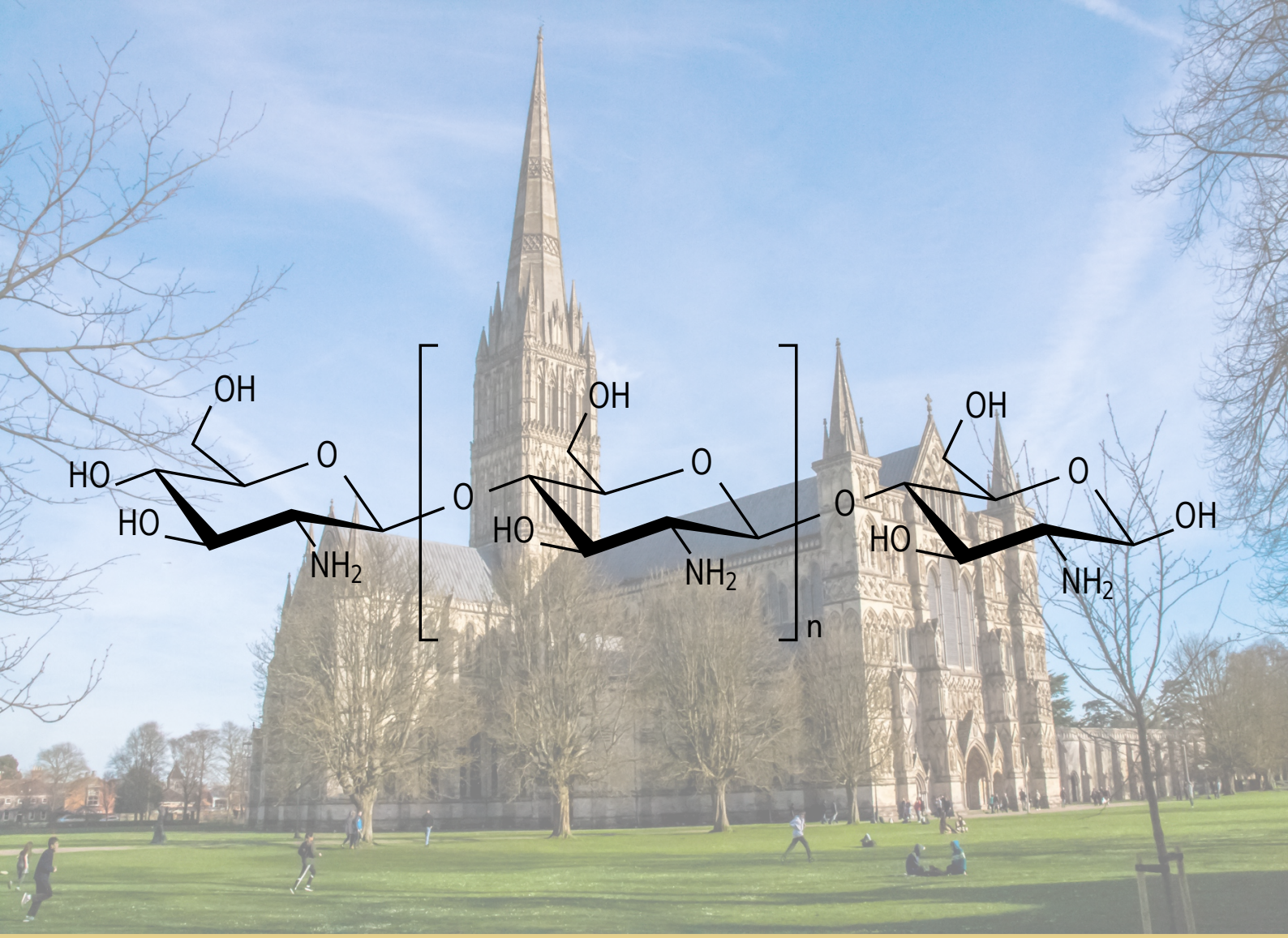

Helena Ferreira Lopes da Silva Santos

Relatório de Estágio de Farmácia Comunitária, e Monografia intitulada "Immunotoxicity of Chitosan Salts and Nanoparticles" referentes à Unidade Curricular "Estágio" sob a orientação de Dr. Fernando Bastos e Doutora Olga Borges, apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas

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Declaração de Autoria

Eu, Helena Ferreira Lopes da Silva Santos, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o número 2012130519, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatório de Estágio e Monografia intitulada "Immunotoxicity of Chitosan Salts and Nanoparticles" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade curricular de Estágio Curricular.

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Coimbra, 13 de Setembro de 2017.

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A eles dedico todo este trabalho.

Contents

Siglas, Acrónimos e Abreviaturas

alum aluminium hydroxide

APC Antigen-presenting cells

cGAS enzyme cyclic-di-GMP-AMP synthase

CS Chitosan

CNP Chitosan nanoparticles

DD deacetylation degree

DC denditric cells

DCI Denominação Comum Internacional

EMEA European Medicines Agency

IFA incomplete Freund's adjuvant

IFNAR IFN-α : IFN-β receptor

Ig immunoglobulin

IL interleukin

IFN-γ interferon gamma

INF-α interferon alpha

INF-β interferon beta

Type I IFN type I interferon

IS immunity system

ISG interferon-stimulated genes

LPS lipopolysaccharide

M1 type classically activated

M2 type alternatively activated

MHC Major histocompatibility complex

mNL mediastinal lymph node

MNSRM Medicamentos Não Sujeitos a Receita Médica

mRNA messenger RNA

MSRM Medicamentos Sujeitos a Receita Médica

MW molecular weight

NALP3 NACHT and LRR and PYD domains-containing protein 3

NF-κB nuclear factor κB

NK natural killer cells

NOD-like receptors nucleotide-binding oligomerization domain-like receptors

NP nanoparticles

ODN oligodeoxynucleotides

OTC *Over the count*

PA *Bacillus anthracis* protective antigen

PDI Polydispersity index

PECs peritoneal exudate cell

RCM Resumo das caracteríticas do medicamento

STING stimulator of interferon genes

SWOT Strenghts Wicknesses Opportunities and Threats

Th T Helper

TLR Toll-like receptors

TNF-α tumor necrosis factor-alfa

Part I

Monograph “Immunotoxicity of chitosan Salts and Nanoparticles”

Resumo

A monografia consiste numa revisão de vários artigos que foram lançados ao longo dos anos que referem e demonstram a capacidade de nanopartículas ou sais solúveis de quitosano interagirem com o sistema imunitário. O quitosano é um derivado desacetilado da quitina, muco adesivo e de fácil produção. Vários artigos demonstraram que interage com células do sistema mononuclear fagocítico, como as células dendríticas e macrófagos, através da ativação do inflamasoma; outros artigos abordam a capacidade das nanopartículas interagirem e ativarem células T, com consequente produção de citocinas e imunoglobulinas. A abordagem é feita também à luz da *guideline* ICH-S8, que prevê testes e estudos que caracterizam a imunotoxicidade.

Palavras-Chave

Imunotoxicidade, Nanopartículas, Quitosano, Inflamasoma, STING, Células T, Citocinas, Imunoglobulinas, *guideline* ICH-S8.

Abstract

The monograph is a survey of chitosan's assays written through time that approach the capability of chitosan nanoparticles or soluble salts to interact with the immune system. Chitosan is a deacetylated derivative of chitin, mucoadhesive and easy to produce. Several articles studied how chitosan interacts with mononuclear phagocytic cells, like dendritic cells and macrophages, through the inflammasome activation; and others approach the T cells activation by nanoparticles, and consequent cytokines and immunoglobulins secretion. The approach will also be made in light of the ICH-S8 guideline, which provides tests and studies for immunotoxicity characterization.

Keywords

Immunotoxicity, Nanoparticles, Chitosan, Inflammasome, STING, T-cells, Immunoglobulins, Cytokines, guideline ICH-S8.

Chapter 1

Purpose

The purpose of this monograph is to review and present immunotoxicity and immunopharmacology studies about Chitosan (CS), including both the polymer and nanoparticles (NP). The interaction with the immunity system (IS) includes the activation of cells, in particular denditric cells (DC), macrophages and lymphocytes, via different pathways.

It is intended also to correlate the characteristics of the CS — that includes parameters like the deacetylation degree (DD), molecular weight (MW) and its purity —, with the effects in the IS and therefore, conclude which characteristics are more relevant for a particular effect on IS. This review will facilitate the application of the ICH-S8 *guideline* for the market authorization of the Chitosan nanoparticles (CNP) as a drug delivery system.

The introduction of the document presents the basic information about CS, NP, and the overall framework. It also gives brief information about the IS and the mechanisms involved with the toxicity, that helps to understand the following chapter, which presents the relevant studies and their conclusions on CS immunotoxicity. In the last chapter, the results are discussed and a global conclusion is presented.

The articles discussed were mostly selected with criteria that I considered essential for a valid conclusion, however some studies presented on this review did not meet the criteria. The criteria include information on the purity or results of the endotoxin test.

All figures were drawn by me.

Chapter 2

Introduction

2.1 Chemical Structure of Chitosan

CS is a polymer with the structure (1-4)-linked-2-amino-2-deoxy-D-glucan (Figure 2.1), and is the N-deacetylated derivative of chitin, a heteropolymer of random distribution of glucosamine and N-acetylglucosamine with 1-4-linkage, that can be found in the exoskeletons of crustaceans and insects, being the second most abundant natural polymer after cellulose [1].

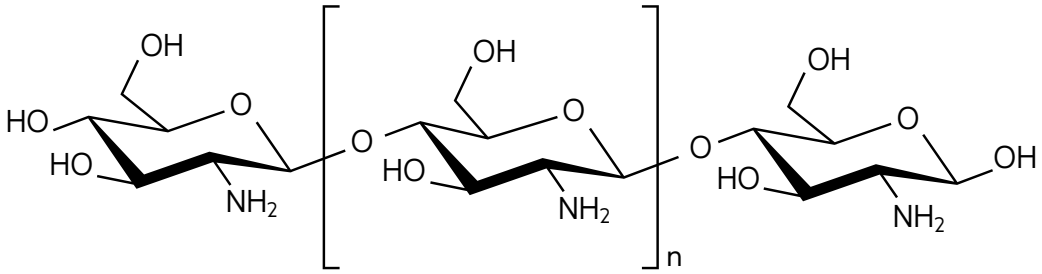

Figure 2.1: Chitosan polymer

CS can be characterized for several parameters like the DD and MW. The deacetylation can result in CS with a DD between 40 % and 98 %, and the MW between 5×10^4 Da and 2×10^6 Da [2]. It is hard to achieve a 100 % N-deacetylation, so CS is the name given when chitin is N-deacetylated to a degree that allows it to be completely soluble in dilute aqueous acids [1].

CS has been reported to be very suitable for nanoparticles, being an attractive excipient for galenic formulations. NP sizes range from 1 nm to 100 nm, and nanomedicine uses them as drug carriers, playing an important role in effective transport and release of the active compounds to the correct site of action. These CNP have many advantages since they are stable, do not require complex preparation methods, have free amine groups for cross linking, have mucosoadhesive properties, are versatile being used for different routes of administration and presents low toxicity [3] [4].

The IS is capable of recognizing nanoscale particles, like viruses. Therefore, NP similar in shape and size to microorganisms can induce effects in the IS, suggesting that the IS may

recognize these materials through conserved pathways. For example, aluminium hydroxide (alum) can immunostimulate the inflammasome, and is being used as a vaccine adjuvant; CpG is a short synthetic oligodeoxynucleotides (ODN) molecule that contains cytosine and guanine triphosphate and acts also as an adjuvant. Therefore, other NP capable to interact with the IS might be able to be used as adjuvants as well, with all of their particularities and advantages [5].

CS toxicity has been studied for a long time, especially as a pharmaceutical excipient. BALDRICK, in the review *"The safety of chitosan as a pharmaceutical excipient", 2010* [6] reported what was already known and what was a motive of concern with the CS's toxicity. But the publications on immunotoxicity the author refers are scarce, dated and superficial, hence the need for this review.

2.2 Immunotoxicity

Immunotoxicity is the name given when there is an interaction between a pharmaceutical compound and the IS. The guideline ICH-S8 *"Immunotoxicity Studies for Human Pharmaceuticals"* [7] from European Medicines Agency (EMEA) defines immunotoxicity as unintended immunosuppression or enhancement. The suppression can decrease the host resistance to infectious agents. The enhancement can exaggerate and induce autoimmune diseases. Immunosuppression or enhancement can be used to modulated the IS for therapeutical purposes, but these effects are not always wanted in other therapies. The guideline focus is restricted to unintended immunosuppression and immunoenhancement.

Moreover, methods to evaluate immunotoxicity are discriminated on the guideline. Standard Studies are: haematology, clinical chemistry, gross pathology, histopathological and organ weights examination. The Additional Immunotoxicity Studies are T-cell dependent antibody response, immunophenotyping, natural killer cells (NK) activity assays, macrophage or neutrophil function, host resistance studies and assays to measure cell-mediated immunity. These parameters should be taken in consideration for future studies with the aim to introduce new drugs or excipients on the market.

Studies on CS and CNP biological activity have been showing that they can actively modulate the body cells [6] [5]. One of the recent interests is the interaction with the IS, not just because of its potential hazard effect, but for its application as a vaccine adjuvant [8] [9].

Most of the studies carried out, with different administration routes, CS and antigen, lack standardization in CS characterization. In fact, very few authors fully characterize the CS, nor specify which product was bought from the supplier or explain with detail the procedure and treatment of CS. Therefore, this makes the interpretation of the results difficult and hard to draw an overall conclusion, since it remains unclear which CS characteristics determine high immunogenicity, or even if the results are not affected due to possible contaminations [10].

2.3 The Immune System

The IS can be generally separated in two groups: the innate system and the adaptive system. The innate IS is the first line of defence against intrusions like microorganisms and nano-organisms, but also other particles with similar sizes, like NP, due to the IS evolution to react to particles that fall within this size range. Some of the main pathways activated by NP are related to cells and mechanisms of the mononuclear phagocyte system like DC and the inflammasome, or other mechanisms like the complement [5].

2.3.1 Mononuclear Phagocyte System

In the Mononuclear Phagocyte System group there are macrophages, DC and monocytes.

Monocytes produce inflammatory cytokines and can differentiate into macrophages or DC. Macrophages recognize many pathogens, have phagocytic mechanisms and induce production of inflammatory cytokines. Macrophages can be classically activated (M1 type), the pro-inflammatory macrophages, activated by interferon gamma (IFN-γ) or lipopolysaccharide (LPS); or they can be alternatively activated (M2 type), primed by interleukin (IL)-4 and IL-13, associated to an anti-inflammatory profile, crucial in tissue remodelling [11].

The DC, when immature, are specialized in antigen-processing and migrate to present them to T cells and B cells; when they are mature and active, they produce a high quantity of cytokines, small proteins that promote cell communication and induce the maturation of the cells. Other than the interaction of antigens that promotes the maturation, these cells are also highly modulated by cytokines. [12].

Several studies showed that CS can trigger an inflammatory reaction, and has been described as an inductor of IL and tumor necrosis factor-alfa (TNF-α) [13] and even able to modulate macrophage polarisation and accelerate macrophage maturation [14].

2.3.2 Immune System receptors

Two of the main receptors that are related to the activation of the innate system are the Toll-like receptors (TLR) and nucleotide-binding oligomerization domain-like receptors (NOD-like receptors). They protect the host against bacteria, virus or particles, since they cover a huge range of possibilities and modifications of the microorganisms. TLR are located in the cellular surface or inside cells in endosome, and can mediate the nuclear factor κB (NF-κB) activation. NOD-like receptors are soluble proteins in the cytosol that protect the cells against intracellular pathogens; both complement each other and synergize the responses to enhance the reaction of the IS, for example, by promoting the assembly of the inflammasome [15].

Besides this type of receptors, there are also nucleic-acid sensors in the cytosol that can detect DNA in the cytosol and induce the production of type I interferon (Type I IFN). Type I IFN induces cell-intrinsic antimicrobial states, modulate innate immune responses promoting Antigen-presenting cells (APC) and NK, and activate the adaptive IS. The most well-defined

types are interferon alpha (INF-α), produced mainly by DC; and interferon beta (INF-β), produced by most cells. They connect to the transmembrane receptor IFN-α : IFN-β receptor (IFNAR) which is composed by IFNAR1 and IFNAR2 subunits, and induce the transcription of interferon-stimulated genes (ISG), that code, for example, for CXCL10 [16].

The basic mechanism of DNA sensing in the cytoplasm is done through the enzyme cyclic-di-GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING). cGAS is a nucleotidyl transferase that produces an endogenous second messenger cGAMP. cGAMP binds to the STING, an adapter molecule located in the endoplasmic reticulum, that, when active, induces Type I IFN production [17]. The mechanism is illustrated in figure 2.2.

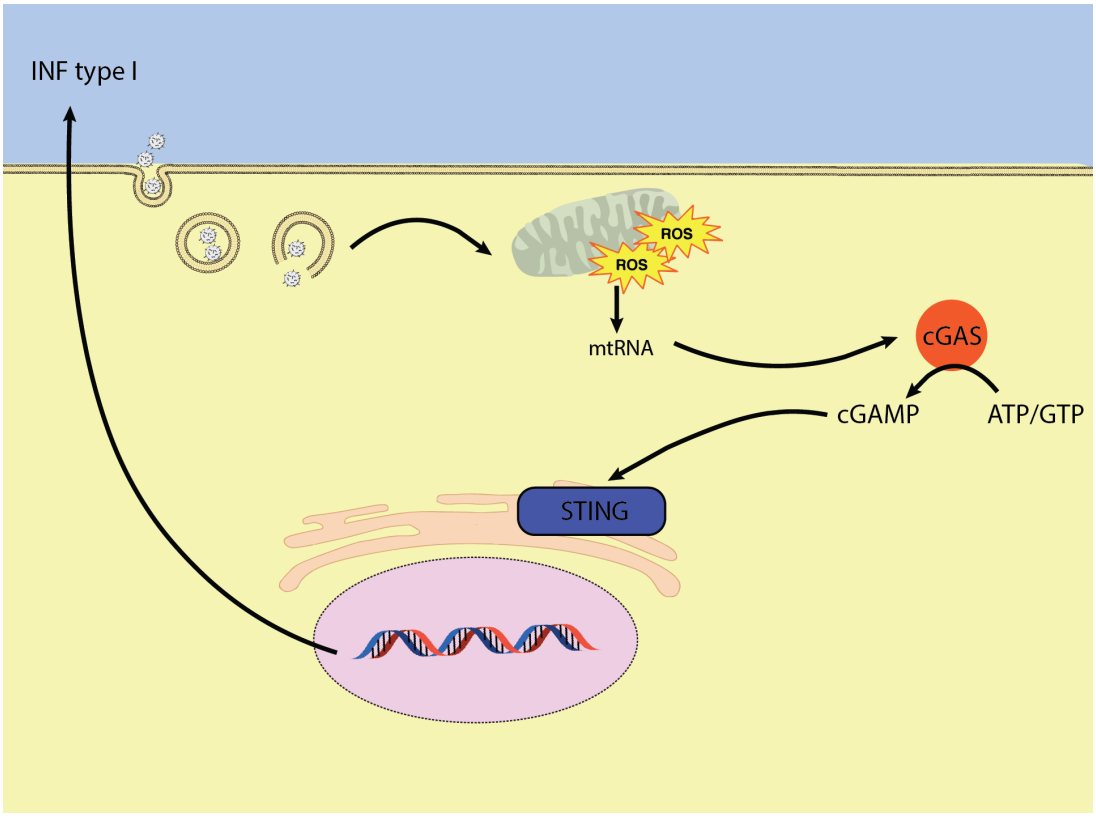

Figure 2.2: cGAS-STING pathway. The NP that reach the cytosol through endossomes induce mitochondrial ROS with consequente release of mtDNA in the cytosol, that activate the cGAS to produce cGAMP, and activates STING, inducing the transcription and production of Type I IFN, that will interact with other cells.

2.3.3 Inflammasome

Inflammasome is a group of cytosolic proteins present in DC and phagocytic cells [18]. When assembled, it triggers proteolytic cleavage of dormant procaspase-1 into active caspase-1, and consequently, the conversion of pro-IL-1β and pro-IL-18 into its mature and active forms. IL-1β is a pro-inflammatory mediator and it is responsible for recruiting immune cells to the site, and IL-18 is important for the production of IFN-γ and potentiation of T cells and NK. Active caspase-1 also induces pyroptosis [19].

There are different inflammasome types, being the NLRP3[i] one of the most documented, since its activation contributes to the maturation of APC such as DC. It has a tripartite structure with NOD-like receptors, the adaptor apoptosis-associated speck-like protein containing a CARD and the protease caspase-1 [19] [11].

A wide range of stimuli were identified to activate the NLRP3 inflammasome, including microbial products, endogenous molecules, and particulate matter [20]; however, two signals are needed. The first signal is generated by TLR-agonist or endogenous molecules such as TNF-α, and they induce the NLRP3 gene and the expression of pro-IL-1β, through the activation of the NF-κB. The ligands do not directly activate the NLRP3; they prime it for activation. The second signal can be triggered by ATP, pore-forming toxins, viral RNA, or particulate matter, and induce the maturation of pro-caspase-1 and activates the rest of the described pathway [21].

The activation of the NLRP3 inflammasome can happen via different pathways that depend on the quality of the second signal. It includes K^+ efflux, Ca^{2+} signalling, ROS, mitochondrial dysfunction, and lysosomal rupture. Particulate matter, like CS, activates NLRP3 mainly through lysosomal rupture, mitochondrial ROS and dysfunction, and K^+ efflux [11] as is represented in figure 2.3.

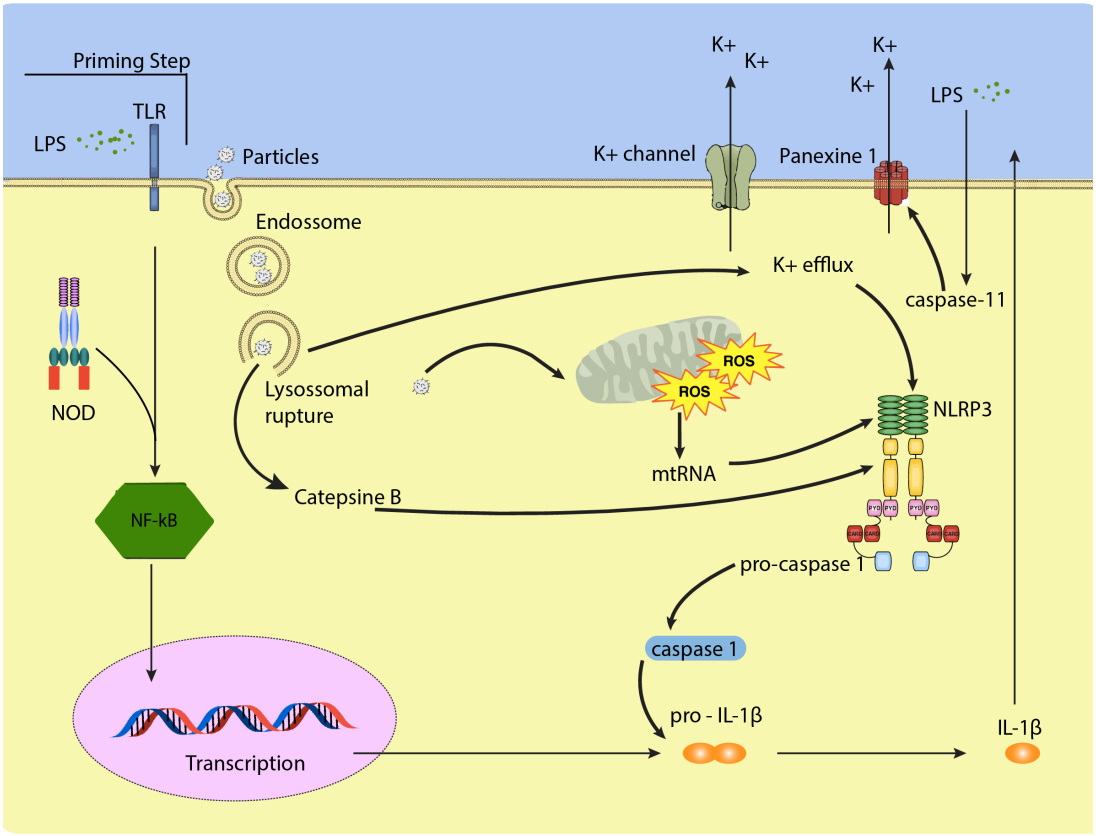

Figure 2.3: Inflammasome mechanisms of activation: priming step, K^+ efflux, mitochondrial ROS, lysosomal destabilization and noncanonical pathway.

[i]The NLRP3 gene encode the NACHT and LRR and PYD domains-containing protein 3 (NALP3) also known by cryopyrin, and is part of the NLR family, with a pyrin domain-containing.

K^+ efflux is related to many kind of inductors. It remains inactive in normal circumstances due to the high concentration of extracellular K^+. However, the efflux, induced in response to most of all NLRP3 stimuli or potassium ionophores such as nigericin, causes a decrease in cytosolic K^+ concentration, and it is sufficient to activate the NLRP3 inflammasome.

Lysosomal destabilization and rupture happens when there is endocytosis of particulate matter that damages the lysosome membrane resulting in the release of cathepsin B, a lysosomal cysteine protease, into the cytosol, that activates the NLRP3 inflammasome. However, there is a lot that remains unclear with this path.

The formation of ROS in the mitochondria due to particles in the cytosol can modify the permeability of the membrane and release mtDNA into the cytosol, that interacts with nucleotide material receptors and activates the NLRP3 inflammasome [19].

LPS from Gram-negative bacteria is a TLR4 agonist that primes the NLRP3, but it can also induce the inflammasome via noncanonical pathway. The LPS has to be phagocyted into the cell and then, it activates caspase-11 that triggers the opening of the pannexin-1 channel through cleavage, which induces the K^+ efflux [19] [22]. Therefore, it is essential that the studies, when analysing these effects, guarantee a high CS's purity through purification methods, or making sure that the supplier provides a pure material (LPS-free), avoiding false positives.

2.3.4 Complement system

Complement system is part of the innate system that, when activated, drives a severe inflammatory response. Part of the effects are anaphylatoxins, opsonins and terminal membrane attack complex. It is constituted by the proteins C1, C2, C3, C4, C5, C6, C7, C8 and multiple C9, and during the activation, they are broken in fragments that are active and continue a cascade.

There are different activation pathways, namely the classical, lectin, and the alternative. In short, the classical pathway goes throw a cascade that begins with the binding between a immunoglobulin (Ig)M or IgG complexed with antigens and the C1 protein. It is cleaved in fragments that cleave C4 and C2, and after that C3 and last the C5. The alternative pathway, which is exemplified in figure 2.4, allows a robust and rapid response, since it starts with a spontaneous cleavage of the C3 protein and the assembly of the C5 convertase, skipping the C1, C4 and C2 cleavage [23].

2.3.5 Adaptive System

The adaptive system is highly specialized in recognizing specific antigens due to immune receptors, that can persist and provide a immunologic memory, having a faster response when re-exposed. It is generally constituted by lymphocytes T and B.

The T cells are the effectors of cellular immune responses and are developed in the thymus. Once developed, they move to secondary lymphoid organs like the lymph nodes

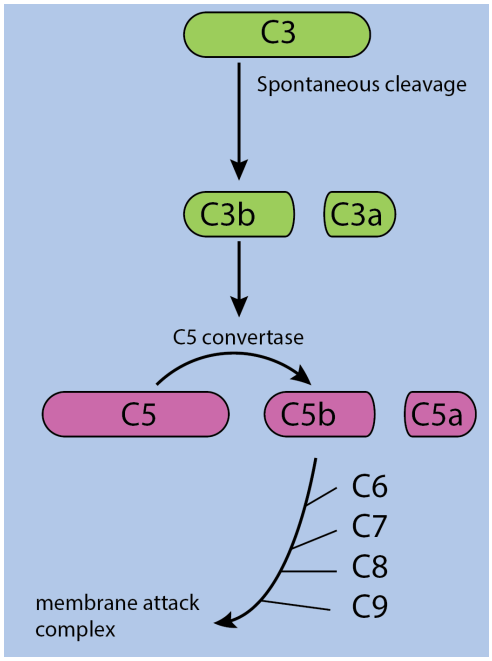

Figure 2.4: Complement alternative pathway

and spleen, where they can interact with the APC already activated. The naive T cells can be $CD8^+$ that interact with Major histocompatibility complex (MHC) class 1 molecules evolving to T cytotoxic cells; and can be $CD4^+$ and interact with MHC class 2 molecules from the APC, evolving to T Helper (Th) cells. Th cells can produce cytokines that activate T and B cells, NK and even the mononuclear phagocyte system; Th can also contact directly with other cells. Depending on the APC and the cytokines, the naive T cells evolve to different sub-effectors.

There are Th1, activated by IL-12, and they secrete IL-2 and IFN-γ. This cytokines are specific for macrophage activation, NK interaction and stimulate the production of IgG2 by B cells. The Th2 are activated by IL-4, and produce IL-4, IL-5, IL-10 and IL-13. They are specific for killing extracellular pathogens, induce eosinophilopoiesis and stimulate antibody production by B cells, like IgG1 and IgE. Th17 are induced by IL-6, and produce a group of five IL-17, potent proinflammatory cytokines with capability to granulocyte recruitment.

Besides Th cells, there are also NK, that are capable of quick and substantial production of cytokines, like IL-4.

B cells mature in the bone marrow where they acquire antigen specificity that, depending on the stimulus, produce different types of Ig, named class-switching [24].

Among the Ig, there is IgM, IgA, IgG and IgE. IgG is one of the most abundant proteins in human serum, and can be separated in subclasses IgG1, IgG2, IgG3 and IgG4. IgG1 responds to soluble protein antigens and membrane proteins, and IgG2 responds to bacterial capsular polysaccharide antigens. The IgG mousse subclasses are different, being IgG1, IgG2a, IgG2b, IgG2c and IgG3. IgA is a mucosal Ig [25]. IgA has high levels in mucosal surfaces and in secretions, having a critical role at protecting mucosal surfaces from toxins, virus and bacteria. IgE has the lowest concentration in the serum and are related to hypersensitivity and allergic reactions, and response to parasitic worm infections as well [26].

Chapter 3

Survey of chitosan's assays

In this chapter, several articles are presented, being all related to the CS interaction with the IS. Three main groups were defined — the first related to DC maturation that includes the NLRP3 inflammasome activation and the Type I IFN pathway; the second related to the secretion of cytokines and Ig, and the modulated response between Th1 and Th2 activation; and a third group where isolated parameters were analysed and do not have a correlation with the other two groups.

3.1 Dendritic cells maturation

The DC maturation is a theme where chitosan is being tested, since these cells are a bridge between innate and adaptive immunity. There are two theories until date, namely the NLRP3 inflammasome and the cGAS-STING pathway, that complement each other and explain the activation of these cells.

The studies that approach the inflammasome activation by CS use the IL-1β levels in the supernatants as a measure of inflammasome activation.

A group of five papers were selected: NEUMANN *et al.* [27], BUETER *et al.* [28] [11], MORI *et al.* [29] and CARROLL *et al.* [30].

NEUMANN *et al.*

The article *"Activation of the NLRP3 inflammasome is not a feature of all particulate vaccine adjuvants"* [27] compares different types of vaccine adjuvants, that includes CNP, alum, cubosomes and incomplete Freund's adjuvant (IFA).

The CNP were prepared by ionotropic gelation, with an average size of 739.0(924) nm, positively charged with a ζ potential of 24 mV and filtered through a 0.2 µm membrane while still a solution, ensuring a bacteria-free product, but not necessary LPS-free.

The particles were tested in *in vitro* generated Bone Marrow DC alone and with a previous incubation with LPS, to confirm whether a priming step with a TLR agonist was necessary to induce the production of IL-1β. From all the tests, only CNP and alum, after the LPS

incubation, were capable to induce the production of IL-1β. Since without LPS, CNP did not induce any production of cytokines, it can be assumed that the potential levels of contaminant LPS are very low.

The CNP were also tested against peritoneal macrophages from the peritoneal cavity, without previous incubation with LPS. In this case, high levels of IL-1β were detected. The alum did not stimulate the macrophages.

An *in vivo* test was performed in mice injected with the particles and a peritoneal lavage was performed. All particles induced an influx of neutrophils, but only with CNP a low amount of IL-1β was revealed.

Finally, a last test with monocytes from human peripheral blood stimulated with CNP and LPS revealed a considerable concentration of IL-1β.

The authors also verified that the activation was dependent on lysosomal destabilization by adding an cathepsin B inhibitor, and consequent reduction of IL-1β.

In conclusion, by comparing different particles in different cells from the mononuclear phagocyte system, the authors conclude that CNP activate the inflammasome via lysosomal destabilization, but it requires a TLR-agonist like LPS to activate the cell first. To justify the production of IL-1β without the activation with LPS by the peritoneal exudate cells, the authors hypothesized that these cells exist in a pre-activated state so that they can react with a pathogen entry. The authors also affirm that, in general, positively charged particles are the ones that can activate NLRP3, since cubosomes and IFA did not induce the IL-1β production.

BUETER *et al.*

The second article, *"Chitosan but Not Chitin Activates the Inflammasome by a Mechanism Dependent upon Phagocytosis"* [28] compares chitin to three different sizes CS: <20 µm, [20 µm , 100 µm] and >100 µm. The CS was obtained from Primex® , has a DD of 76% and it was treated with NaOH for bacterial endotoxin destruction.

The authors tested the samples on Bone Marrow-derived macrophages, previously incubated with LPS. CS stimulated IL-1β, but chitin did not produce a relevant amount.

Tests were made also with macrophages from NLRP3$^{-/-}$ mice to check if the inflammasome activation was NLRP3-dependent. The supernatant had a big reduction on the levels of IL-1β, comparatively to the previous test.

On the third test, they compared the three CS's sizes, being the smallest size the one that induced more IL-1β, and the biggest size the one that induced the fewer amount.

Since the sizes tested were not precise by being a group of sizes, they also coated beads with 3 µm and 50 µm with chitosan and chitin. Only the beads with 3 µm were phagocytosed, and just the chitosan-beads stimulated a strong IL-1β response. Neither one of the chitin-beads produced a response, nor the 50 µm chitosan-bead.

They also tested soluble CS[i] after LPS incubation, but it showed weak capability to activate the inflammasome, with less then 20 % of IL-1β from that seen with insoluble CS.

Since the inverse association of size of the CS particles with inflammasome activity suggests that phagocytosis is necessary for the activation, a last test was performed, measuring the IL-1β after a treatment with cytochalasin D, an inhibitor of phagocytosis. Indeed there was a significantly reduction of the amount for the CS. However, they tested for soluble nigericin, an other NLRP3 activator, and the levels were not affected. This means that, for CS, phagocytosis is needed to activate the inflammasome.

By performing these tests, the authors could conclude that chitosan, but not chitin, potently stimulates the production of IL-1β after an incubation with a TLR-agonist like LPS via a size-dependent mechanism, that includes a phagocytic step. They also conclude that soluble CS does not activate the NLRP3 inflammasome. This overall conclusion agrees with the previous article, since chitin has negative charge and did not activate the inflammasome, and also the phagocytosis is required for a lysosomal destabilization.

Later, the same investigators proceeded to new tests and wrote *"Spectrum and Mechanisms of Inflammasome Activation by Chitosan"*[11].

Using CS with the same specifications and extensively purified as previously, they tested peripheral blood murine cells and induced their maturation to M1 type, M2 type or intermediate phenotypes macrophages. After a priming step, CS promoted the secretion of IL-1β in all of them, an more intensively on the M1 type. They also searched for IL-18, an inflammasome-related cytokine, and the release was stimulated by CS.

They also verified that the IL-1β is NRLP3-dependent in DC and peritoneal macrophages. They tested which pathway of the NRLP3 inflammasome could be implied. They inhibited the K^+ efflux, and the level decreased significantly. They also inhibited the mitochondrial ROS and there was a significant reduction in IL-1β release. Finally, the authors tested the role of lysosomal destabilization, and, by inhibition, they reduced the IL-1β levels once again. Therefore, their data suggest that lysosomal destabilization, mitochondrial ROS and K^+ efflux is needed for an optimal NLRP3 inflammasome activation and IL-1β secretion.

Finally, they tested the supernatants for 22 cytokines in the unprimed cells, and CS did not induced any one of them.

Concluding, CS induces the production of IL-1β, among with IL-18, in primed cells with a TLR-agonist, with a stronger response in M1 type macrophages, via activation of the NLRP3 inflammasome thought the lysosomal destabilization, mitochondrial ROS and K^+ efflux pathways. An unprimed cell does not secret any cytokine in the presence of CS.

[i] They solubilized chitosan by dissolving it in dilute acetic acid.

MORI *et al.*

A third study, *"The vaccine adjuvant alum inhibits IL-12 by promoting PI3 kinase signaling while CS does not inhibit IL-12 and enhances Th1 and Th17 responses"* [29], used a CS salt that is soluble in water, DD between 75 % and 90 %, with levels of endotoxins $\leq$100 EU/g, and obtained from Novamatrix® [ii].

The purpose of this study was not only to verify the IL-1β production by CS, but to test if it does not inhibit the production of IL-12, like alum does, another cytokine produced by DC and macrophages. The authors used bone marrow-derived immature DC from mice.

The CS salt, by itself, did not promote significant secretion of IL-1β. But with LPS, not only did it optimally promote the secretion of IL-1β, but also it did not inhibit LPS-induced IL-12 secretion. This result contradicts Bueter *et al.* since a soluble CS stimulated de production of IL-1β.

The authors tested also the need for phagocytosis to activate the inflammasome, by blocking with cytochalasin D like Bueter *et al.*, and concluded the same.

They went further and tested others TLR agonists, and found out that CpG, a TLR9 agonist, can trig the first step for the inflammasome activation and induced the secretion of IL-1β, being the combination of CS and CpG the optimal formulation for inducing IL-12, IL-23, and IL-6 secretion by DC.

In conclusion, the authors reinforced the idea that CS, even though being a soluble salt, is capable of inducing the production of IL-1β with a TLR-agonist, and requires endocytosis, even when solubilized. Also, CS does not inhibit IL-12 secretion induced by LPS or CpG.

CARROLL *et al.*

In the study *"The Vaccine Adjuvant Chitosan Promotes Cellular Immunity via DNA Sensor cGAS-STING-Dependent Induction of Type I Interferons"* [30], the authors approach the IS modulation via a different pathway. They use also the CS salt Protasan® ultrapure ,with endotoxins $\leq$ 100EU/g, from Novamatrix®, like Mori *et al.* [29].

The study focused on the capability of chitosan to drive cellular immunity through Type I IFN mechanism. They started with *in vivo* studies. They immunized wild-type mice and IFNAR$1^{-/-}$ mice with a hybrid antigen and CS. The wild-type had an enhancement in the production of IFN-γ with CS-hybrid antigen, by mediastinal lymph node (mNL) and $CD3^{+}$ $CD4^{+}$ but not $CD8^{+}$, limited production of IL-17 or Th2-associated cytokines, IgG2c and IgG1; only the IgG1 was not compromised at the IFNAR$1^{-/-}$ mice.

The authors tested also the capability of CS to activate the DC against LPS and CpG as positive controls. CS enhanced equally the surface expression of CD40 and CD86 by DC, but without secretion of pro-inflammatory cytokines such as IL-6 and IL-12. They ruled out the involvement of TLR4 since they used mice defective in it. Therefore, CS promoted DC maturation via a different pathway then that involving TLR4.

[ii] Protasan® ultrapure chitosan salt (CL213).

Since the two results were pointing to a Type I IFN mechanism, the authors first checked if CS was capable to induct the transcription of *Ifna* and *Ifnb* messenger RNA (mRNA). CS was capable to induce the expression of both mRNA, but only *Ifnb* was independent of IFNAR, as they showed with mice without the gene for IFNAR. CS promoted INF-β, that resulted in the transcription of the ISG chemokine CXCL10.

Not only CS needs IFNAR to produce INF-α, but it was proven also that CS needs IFNAR for the DC maturation, by testing mice with absence of the *Ifnar1* gene.

They tested the pathway via STING, and mice without the gene did not produce any *Ifnb* when stimulated with CS. Moreover, the secretion of CXCL10, INF-β was absence. That did not happen with the control of LPS.

Upstream of STING there is cGAS. The response with CS was also abrogated with mice without the cGAS gene, but not the response with LPS. Once again, the secretion of CXCL10 and INF-β was compromised, and the enhancement of CD40 expression on DC inhibited.

All the tests were repeated *in vivo* and had the same conclusion. CS with the hybrid antigen, requires cGAS and STING to induce IFN-γ by mNL and $CD3^+$ $CD4^+$ peritoneal exudate cell (PECs), and IgG2c.

Since the authors read about the NLRP3 inflammasome activation by CS, they also tried to induce a cellular immunity response in *Nlrp3*$^{-/-}$ mice. As expected, the capability to induce IFN-γ was highly decreased. However, neither IgG1 nor IgG2c had a response significantly reduced compared to the wild-type mice. This shows a complementarity between NLRP3 inflammasome and cGAS-STING.

To enrich the findings, they also tested a mucosal administration of CS with a different antigen, and they reached to the same conclusions. Therefore, with two different administration routes and two antigens, the authors reached the same result with wild-type mice and mice without the STING gene.

Since STING can be activated by genetic material, the authors speculated if CS was also inducing mitochondrial stress, specially mitochondrial ROS and the release of mtDNA into the cytosol. Indeed they observed it as a response to CS and it was comparable to the results of rotenone, the positive control. By adding the antioxidant MitoTEMPO, the CS-induced secretion of INF-β and CXCL10 was suppressed, but not with LPS or CpG, indicating a specificity for CS. Last, with an inhibitor of mitochondrial permeability transition pore, cyclosporin A, they inhibited the released of mtDNA into the cytosol, and as they suspected, the secretion was suppressed. The CS-induced upregulation of CD40 was also inhibited, but not with CpG.

After putting together all the information the authors conclude that CS-induced cellular immunity happens through a mitochondrial damage, realising the mtDNA into the cytosol. This event activates the cGAS-STING and the Type I IFN pathway.

3.2 Lymphocytes responses

Although there is no clear explanation for CNP mechanism of action related with lymphocytes, there are results worth mention. A group of ten papers was selected: MORI *et al.* [29], WEN *et al.* [31], WU *et al.* [32], ZAHAROFF *et al.* [33] [34], LIU *et al.* [35], FARACE *et al.* [36], BENTO *et al.* [37], BORGES *et al.* [38] and ZHAO *et al.* [39].

MORI *et al.*

Although the article *"The vaccine adjuvant alum inhibits IL-12 by promoting PI3 kinase signaling while CS does not inhibit IL-12 and enhances Th1 and Th17 responses"* [29] previously presented focused on the NLRP3-inflammasome, the authors also did some research on the antibody response, using the same CS .

They tested the ability to enhance an antigen-specific IgG2 antibody responses. When CS was added with an antigen subcutaneously, an enhanced antigen-specific response was detected with IgG1, IgG2a, and IgG2b. However, this response was not dependent on NLRP3 inflammasome, since NLRP3$^{-/-}$ mice had a comparable response.

They tested *in vivo* the potential of CS-CpG as an adjuvant to promote Th1 and Th17 responses. CS-CpG promoted an antigen-specific IFN-γ response in PECs and enhanced antigen-specific IL-17 response in restimulated spleen, lymph node cells, and PECs.

In conclusion, besides the NLRP3 inflammasome results, the authors found out also a lymphocyte response in the presence of CS, promoting a Th1 and Th17 response with secretion of IFN-γ and IL-17.

WEN *et al.*

In the article *"Chitosan Nanoparticles Act as an Adjuvant to Promote both Th1 and Th2 Immune Responses Induced by Ovalbumin in Mice"*[31], the authors tested CNP with a DD of 95 %, a diameter of 83.66 nm, a ζ potential of 35.43 mV and sterilized by passing through a 0.22 µm Millipore filter. All the tests were run for the antigens of ovalbumin and had a control of ovalbumin, a saline control, and a positive control (QuilA).

CNP was tested for splenocyte proliferation, and the results show that it has a higher response compared to the controls. The same happened with NK where CNP enhanced their killing activity in mice immunized with ovalbumin.

Cytokines in the supernatant were also tested, and CNP induced the production of IL-10, a Th2 type immune response; and IL-2 and IFN-γ, a Th1 type immune response, suggesting the role of CNP in Th1 and Th2 activity. After, they analysed the mRNA expression from the splenocytes and confirm the Th1 and Th2 activity with high levels of IL-10, IL-2 and IFN-γ mRNA, suggesting the up-regulation of these cells.

Besides cell response, they also checked humoral response, and analysed the serum antibody response. The ovalbumin-specific antibodys quantified were IgG, IgG1, IgG2a and IgG2b.

CNP caused significant enhancements in ovalbumin-specific IgG2a and IgG2b, a Th1 immune response, and less but still higher results for the IgG and IgG1, a Th2 immune response.

These results show that CNP can modulate directly the lymphocytes, not only on their proliferation, but also on their secretions. Even more, CNP stimulated a balanced Th1/Th2 immune response.

WU *et al.*

In the article *"A Novel Chitosan CpG Nanoparticle Regulates Cellular and Humoral Immunity of Mice"*[32], the authors test the capability of CpG encapsulated in CNP to enhance humoral and cell response. The CNP have a diameter of 45 nm and a ζ potential of 25.6 mV. There is no reference for endotoxins treatment or purity of the CNP. The controls from the test were saline solution, and naked CpG with five times more CpG then the CNP-CpG.

They tested the changes of the number of immune cells *in vivo* with vaccinated mice intramuscularly or orally, and the CNP-CpG enhanced the levels of the cells, specially the lymphocytes and monocytes, compared to the saline control. The result agree with Wen *et al.* However, the results from the monocytes where not significantly different from the naked CpG, even though higher then the control. None of them had a big response of neutrophils.

The authors quantified the levels of IL-2, IL-4 and IL-6 in sera of inoculated mice. The results with the CpG-CNP were all higher than the control, but they were not different from the naked CpG, which means that the CNP did not have any influence. This contradicts the previous articles since CNP are expected to induce the levels of IL-2 and IL-6.

The content of IgG, IgM and IgA where significantly higher then the saline, but only the increase of IgG was higher with CNP-CpG. This was also expected since it agrees with Wen *et al.* The CNP addiction did not enhanced the IgM and IgA.

Between the oral and intramuscular vaccinated mice, there were no significant differences in any level of the parameters measured.

Despite these results, we cannot have a right conclusion on CNP for two reasons: first, there is no information about the purity so we cannot conclude if the results are specific for CNP; and second, there are no tests for CNP alone. Therefore, we can not conclude if the enhancement from the CNP-CpG is not just a technological upgrade that potentiated the CpG effects by protecting against degradation, or a endotoxin-inducing.

However, the negative results might be considered since the addition of CNP did not changed the response, which means that, even with any contaminant, there was no response. Therefore, we can conclude that CNP does not induce IgM and IgA response. The results that are contraindicative — the IL-2 and IL-6 levels — need further research. The low amount of neutrophils might be explained by the inexistence of chemotaxis.

ZAHAROFF *et al.*

In the article *"Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination"*[33], the authors test a CS soluble from Novamatrix®, the Protasan® G213, with a DD of 75 %-90 % and a level of endotoxins $\leq$100 EU/g.

They vaccinated mice subcutaneously with β-galactosidase and CS or saline solution. The proliferation of $CD4^+$ splenocytes was significantly higher with CS. The levels of IgG against β-galactosidase were significantly higher with CS, especially IgG1 and IgG2a, implying a mixed Th1 and Th2 response. These results are similar to the previous articles.

The mice were challenged with β-galactosidase a week later, and the ones vaccinated with CS symptoms of a robust cell-mediated immune response.

The mice were dissected and the size of the lymph nodes was bigger in those vaccinated with CS. The leukocytes in lymph nodes increased more than 67 %, specially NK and $CD11b^+$.

In conclusion, CS soluble can induce an humoral and cellular response against non-immunogenic molecules, even when administrated via subcutaneously.

Later, the same team wrote *"Chitosan solution enhances the immunoadjuvant properties of GM-CSF"*[34], where they tested the response against inactivated Human Influenza A strain PR/8 purified virus, administrated subcutaneously. They registered a antigen-specific proliferation of $CD4^+$ splenocytes, but no changes in the levels of IgG.

LIU *et al.*

The article *"Therapeutic efficacies of chitosan against Pneumocystis pneumonia of immunosuppressed rat"*[35], the authors test the effects of CS in immunosuppressed rats as a treatment, not a vaccine, for *Pneumocystis* pneumonia.

The CS was bought from Sigma-Aldrich and it was solved in acetic acid and water with pH adjusted to 5.3 with NaOH, and sterilized at 121 °C for 15 min; it was injected in the tail vein of the rat for seven days. There were several rat groups: the normal group with immunocompetent rats not infected with *Pneumocystis carinii*, the CS-normal group with immunocompetent rats, uninfected and injected with CS, the model group with immunosuppressed infected rats, CS group with immunosuppressed infected rats and injected with CS, and acetic acid group, with immunosuppressed infected rats and injected only with the acid.

The results showed an increment of $CD4^+$, and IL-10 and IFN-γ serum concentration with CS, compared to the model and acetic acid groups; and a decrease of $CD8^+$, macrophages and TNF-α. There was also a decrease of *Pneumocystis pneumonia* HSP70 mRNA, that represent and healing due to the IS activation with CS.

Once again, now as treatment, CS modulated the immune system and had promising results.

FARACE *et al.*

In the article *"Immune cell impact of three differently coated lipid nanocapsules: pluronic, chitosan and polyethylene glycol"*[36], the authors test CNP with 340 nm *in vitro* on peripheral blood mononuclear cells that include T, B, NK cells and monocytes, from healthy donors.

There is no more information on the CNP characterization, so we don't know the DD or endotoxins level. Therefore, the results must be analysed carefully.

They first tested the uptake by $CD3^+$ T cell and $CD14^+$ macrophages, that was well succeeded. However, CNP induced apoptosis in some of the T cells and monocytes. It also enlarged the diameter of monocytes and activated as well CD25 T cells and CD96 T cell and monocytes.

Later, they searched for cytokines secretion and compared against LPS and negative control. They detected high levels of IL-6, IL-10, TNF-α and IFN-γ. They also detected levels comparable to the levels of LPS of IL-4, IL-12, and lower the LPS levels of IL-13. They did not detect IL-2 in neither one.

These results reflect a macrophage, Th1 and Th2 response. However, since we do not have a clear notion on the purity of the CNP, some of these results can be due to a contamination, especially the ones where the levels of IL induce by LPS and CNP are similar. Even more, the results from other articles so far did not have a positive response on the cytokines secretion without previous incubation with LPS. It might be justified, besides the contamination with LPS, with a pre-activated state of the cells, like Neuman *et al.* suggests.

BENTO *et al.*

The article *"Development of a novel adjuvanted nasal vaccine: C48/80 associated with chitosan nanoparticles as a path to enhance mucosal immunity"*[37] approaches a CNP with a 95 % DD, a size with an average size of 396.2 nm, a ζ potential of 21.59 mV and purified.

First, the authors tested if the CNP would activate human mast cell line, by measuring the β-hexosaminidase release as a marker for cell degranulation. Indeed, CNP activated it, with significant levels of β-hexosaminidase.

Next, they tested the levels on IgG antibodies against the *Bacillus anthracis* protective antigen (PA) combined with CNP. Mice were intranasally immunized, and PA-CNP significantly increased the anti-PA IgG, namely IgG1 and IgG2c. However, it did not induce serum levels of IgE.

The authors also tested the IgA in the mucosae, namely the nasal, vaginal and fecal. Fecal pellets were collected and showed a high level of IgA. The other two did not have such a high quantity of IgA.

Finally, cytokines were quantified in re-stimulated splenocytes, and CNP induced high levels of IL-17, IL-22, IL-4 IL-10, IFN-γ and IL-2.

In conclusion, in this article not only the authors presented the activation of mast cells as a mechanism that may contributes to the immunotoxicity of CNP, but they reinforced the

idea that CNP induce Th1, Th2 and Th17 response, and the production of IgG. For the first time, CNP induced IgA, and it was also proved that CNP does not induce IgE.

BORGES *et al.*

The article *"Induction of lymphocytes activated marker CD69 following exposure to chitosan and alginate biopolymers"*[38] uses cell spleen from mice to analyse the impact of soluble CS in it. They authors tested a CS with a 95 % and filtered with a 22 µm pore.

The lymphocytes had an enlargement in both forward and side scatter, and cellular granularity. It affected $CD4^+$, $CD8^+$ and B cells.

Later, they tested the capability to induce the expression of CD69 in the spleen lymphocytes. All of them expressed it greatly with CS. However, when they tested the proliferation, CS did not promote the proliferation of the splenocytes. But CS did not inhibit it also, since CS was tested combined with other particles that induced the proliferation and did not reduce the levels.

ZHAO *et al.*

In the article *"Preparation and Efficacy of a Live Newcastle Disease Virus Vaccine Encapsulated in Chitosan Nanoparticles"*[40], the authors, among other things, measure the levels of IgA produced when CNP is administrated orally to chicken. The CNP have a DD equal to 80 %, average size of 371 nm, and where filter through a 0.22 µm pore before the formation of the CNP.

The CNP were administrated orally, and then, they analysed the intestinal mucus from the duodenum lumen after a week. The particles did not have significantly increase of IgA antibody concentration compared to the saline.

3.3 Others

Other articles reported interactions between CS and the immune system that are not include in the previous articles. CHUA *et al.* [9] approached the capability of DC to phagocyte and transport NP and microparticles to lymph nodes. MINAMI *et al.* [41] tested the capability of CS to activate the complement system. LEE *et al.* [42] tested CNP as an anti-inflammatory particle, by measuring the levels of eosinophils in lungs.

CHUA *et al.*

In the article *"CS Microparticles and CNP as Biocompatible Delivery Vehicles for Peptide and Protein-Based Immunocontraceptive Vaccines"* [9], the authors compare two molecule sizes and their capability to be internalized by DC and also to induce an immune response against luteinizing hormone-releasing hormone, a nonimmunogenic peptide antigen.

They used CS with low MW, DD 75 %-85 %, but no information about the purity or the endotoxin level. The CNP have a size within 113 nm-213 nm, the CS microparticles have a size within 1.62 µm-2.58 µm.

The authors demonstrated that the up-take by bone marrow DC is dependent on concentration with fluoresceinated particles, and both sizes were well up-taken, with CNP at a higher rate. This agrees with Bueter *et al.* that used beads with the same size as the microparticles.

It was tested *in vivo* if the uptake by DC resulted in the transportation of the particle to the draining lymph nodes, where they would be presented to naive T cells. Both of them where carried, with CNP-associated cell travelling faster than microparticles-associated cell.

The antibody levels were much higher than the control with the antigen in saline solution, representing a robust antibody response against the self-hormone.

Although the results suggest that the use of CNP or microparticles can be useful as a vaccine adjuvant to enhance the immune response against poorly immunogenic molecules, we cannot conclude more based only on this article, since there is no information on the purity. But we can conclude that indeed, once again, the micro and nano size are internalized and interact with DC, and are transported to the draining lymph nodes to activate T cells.

MINAMI *et al.*

The article *"Chitin and chitosan activate complement via the alternative pathway"*[41] approaches the imunotoxicity from another point of view. A CS with a DD of 82 %, sterilized by ethylene oxide gas and size <3 µm, was suspended in water and tested *in vitro* in human blood serum from healthy donors.

The studies where made against a positive control. The results show an acute decrease of the C3 level, no changes in the levels of C4, and again a decreased serum C5 level, similar to the positive control.

This kind of response is typical from an activation of the alternative pathway, showing a anti-inflammatory reaction.

LEE *et al.*

"Thiolated chitosan nanoparticles enhance anti-inflammatory effects of intranasally delivered theophylline"[42] approaches CNP whit 90 % DD, diameter of 220 nm, and a ζ potential of 22.7 mV. There is no information about the purity or endotoxins level.

They tested the capability of CNP to be anti-inflamatory, by decreasing the number of eosinophils. The intranasal administration of CNP to OVA-challenged allergic mice did not reduced the levels of eosinophils, nor induced apoptosis in inflammatory cells from lungs. CNP did not produce any anti-inflammatory effect.

Chapter 4

Discussion

CS has been tested for many years as a potential vaccines adjuvant or as drug delivery system for disease treatment purpose. It is biodegradable, mucosoadhesive, non toxic and easy to obtain. The potential for immunoenhancement of CS has been a main concern and a motive of investigation, and many results have been presented so far.

The introduction of the ICH-S8 guideline in 2006 came as marker for the importance of the immunotoxicity and to guide through the tests that evaluate it. Even though none of the tests post-guideline refer it, some of the assays can be related with the tests proposed on the guideline.

This review looked through many articles, and 16 met the criteria to be included in the analysis.

Five articles work in the NLRP3 inflammasome activation — [28] [11] [30] [27] [29] — and all of them propose the NLRP3 activation as one of the main paths that CS induce immunostimulation. Different types of CS were used, namely CNP, soluble salts and microparticles. All of them specify the purification of the material, and have good controls. So, the results should be considered valid. All the results concluded that CS activates the NRLP3 inflammasome and that is how it induces the secretion of IL-1β, but it requires a priming step with a TLR-agonist. This is an exception for murine peritoneal macrophages collected from peritonial cavity that expressed levels of IL-1β without the priming, suggesting that they are in pre-activated state. Further tests should evaluate this situation.

Not only the different authors were able to solid confirm this activation, but also were able to explain the mechanisms by which CS activates the NRLP3 inflammasome. Phagocyte is required, and the K^+ efflux, mitochondrial ROS and dysfunction, and lysosomal rupture are all three pathways that CS activates. The best results were achieved with smaller particles, suggesting that the phagocytosis is a core step.

Different cells from different species *in vivo* and *in vitro* were tested among the five studies, and CS induced the NLRP3 inflammasome in macrophages, DC and monocytes, not only collected from the bone marrow, but also from peripheral blood, covering the mononuclear phagocyte system.

All the results were able to induce IL-1β, and Bueter *et al.* searched also for IL-18, an

expected cytokine released due to inflammasome activation. It detected significant levels after the prime step.

There are not many discrepancies in the results, however, BUETER *et al.* [28] [11] said that a soluble CS was not able to induce the NLRP3 inflammasome, but MORI *et al.* [29] and CARROLL *et al.* [30] were able to induce strong results with a salt. Both of them bought the CS from Novamatrix®, and BUETER *et al.* [28] [11] dissolved the CS in acetic acid. The procedure might be the cause for these results, as the other was a chloride salt soluble in water, and no acetic acid was used. Further studies are needed to evaluate which CS salts can indeed induce NLRP3 activation.

Another investigated pathway for the IS activation was the cGAS-STING pathway [30]. The authors worked with a soluble CS purified and were able to correlate the effect of CS *in vitro* and *in vivo* by two administration routes, with the secretion of Type I IFN that activates Th1 cells. They do not revoke the NLRP3 inflammasome theory, but prove that the inflammasome interacts mutually with the accGAS-STING pathway. They proved too that CS induces mitochondrial ROS and that is the way that accGAS-STING pathway is activated, and not only the NLRP3.

They detect Type I IFN levels, specially INF-β in unprimed cells. Even though BUETER *et al.* 2014 [11] tries to detected a huge range of cytokines in unprimed cells induced by CS, they do not test Type I IFN. Therefore, these information complement and do not cancel each other.

CARROLL *et al.* [30] detect IFN-γ and IgG2c, which proved that the cGAS-STING pathway successfully activated Th1 cells. But they do not detect high levels of Th2 cytokines or IL-17. It might be due to the IFN-γ that suppresses Th2 and Th17 cell differentiation.

They were not the only ones detecting cytokines produced by cells. Many of the studies focused on the lymphocytes activation and secretion [36] [31] [32] [35] [37]. Cytokines secretion was controversial. Although some cytokines, when searched, were detected, others were not always detected.

The cytokines that were concise were IFN-γ and IL-10, that suggest a Th1 response, and monocytes or a Th2. IL-12 was detected by FARACE *et al.* [43], and MORI *et al.* [29] concluded that CS does not inhibit its secretion, and enhances its production with the optimal concentration. The cytokines IL-13 [36] and IL-22 [37] were analysed only once. These results could represent a balanced Th1/Th2 response. FARACE *et al.* [43] do not refer a purification method and so the IL-13 might be or not due to the CNP.

The controversial cytokines were TNF-α, where LIU *et al.* [35] noticed a reduction in the levels but FARACE *et al.* [43] had an increment; FARACE *et al.* [43] and WU *et al.* [32] did not detect IL-2 in considerable levels but WEN *et al.* [31] and BENTO *et al.* [37] did; IL-4 was detected by FARACE *et al.* [43], BENTO *et al.* [37] but not by WU *et al.* [32]; IL-6 was again detected by FARACE *et al.* [43] but not by WU *et al.* [32]; and last, IL-17 was detected by BENTO *et al.* [37] but not by CARROLL *et al.* [30] Those facts may be related to the antigen used in each study or with the mice specie, which were different.

Moreover, since some cytokines inhibit others, it is possible that the quantities of CS or the sample of cells used can reflect a more or less strong cytokine induction. However, it is clear that CS induces Th1, Th2 and Th17 responses.

Not only cytokines where analysed, but antigen-specific Ig levels were quantified. Both Th1 and Th2 IgG were detected in the majority of the studies, but was dependent on the antigen used. In every study, CS was a good adjuvant for different antigens inducing a more strong antigen-specific antibodies production, like IgG and IgA. It is important to highlight that it did not induce IgE production, which means that it is unlikely for CS to induce allergic reactions.

Besides lymphocytes, other cells recruitment were analysed. Neutrophils did not changed more with CS then with the controls [27], nor the eosinophils decreased [42]. The article from BENTO *et al.* [37] was the only one that analysed the effects on mast cells, concluding that CS induce their activation, being a promising way to IS activation. NK were detected by ZAHAROFF *et al.* [33].

More elucidations on the CS mechanism where discovered. CHUA *et al.* [9] discovered that the uptake by DC was concentration-dependent, and that the cells with the CS particles successfully transported them to the lymph nodes to activate naive T cells, being the smaller particles faster.

MINAMI *et al.* [41] revealed another way CNP activate the innate system with the complement activation via the alternative pathway.

From all the tests, some of them comply with the ICH-S8 guideline. This means that the tests suggested on this guideline are already being done by some researchers with the aim to evaluate the possible immunotoxicity. For instance, NEUMANN *et al.* [27] determined microscopically the total and differential leucocytes levels, which is a standard toxicity study. ZAHAROFF *et al.* [33] made a gross pathology by analysing the size of the lymph nodes, and noticed an increased in size. The T-cell dependent antibody response was the most common test. However, many studies still need to be performed in order to fulfil the ICH-S8 characterization, following its indications, and be able to fully understand CS properties as a modulator of the IS.

A compilation of the articles main information can be found outlined and summarized in a table in the appendices.

Chapter 5

Conclusion

CS showed to be a modulator of the IS, either innate or adaptive. There are still many studies to perform to reach a final conclusion, and they should be targeted to the ICH-S8 guideline. Another major problem is the inconsistency of the CS characterization, that must be fully performed in order to have valid results. It should include the supplier and the specific product bought, origin, DD, MW, ζ potential, Polydispersity index (PDI), viscosity, particle size, surface and shape, and the purification method or endotoxins levels.

Four of sixteen articles did not mention the endotoxins levels or a method used to treat the CS - LEE *et al.* [42], CHUA *et al.* [9], WU *et al.* [32] and FARACE *et al.* [36]. Therefore, their results might not be as accurate as the others.

In summary, CS salts or CNP must be phagocyted by the mononuclear phagocyte system, being small sizes <3 µm ideal for this immuneenhancement, and through multiple mechanisms induce an innate response that activates Th cells with consequent cytokines release and Ig production.

These effects are wanted for CS if it is used as an adjuvant, but may not be when combined with other proteins like insulin, for example. Its use for cronical diseases might not be also ideal, as the person would be exposed for a long time to a imunoenhancer. The ideal use would be for acute situations, as long as it induces reversible reactions and short time effects.

Parte II

Relatório de Estágio de Farmácia Comunitária

Resumo

Este documento reúne para além da monografia, o relatório de estágio de farmácia comunitária.

O relatório de farmácia comunitária segue o formato de uma análise SWOT fundamentada, como indicado nas Normas Orientadoras. O estágio decorreu em Aveiro, de Abril a Agosto. Pude acompanhar o trabalho que se exerce na farmácia, não só em termos encomendas e atendimento ao público, mas também nas decisões de gestão e organização da farmácia.

Em anexo encontra-se o relatório do estágio de farmácia hospitalar, feito ao abrigo do programa Erasmus, em Salisbury, no Reino Unido. O relatório segue o movimento do medicamento no hospital, tal como pedido pela instituição de acolhimento.

Palavras-Chave

Farmácia Comunitária, Encomendas, Atendimento, Aconselhamento, Prescrição Médica, Parâmetros Bioquímicos.

Abstract

This document covers also the Community Pharmacy Placement Report, besides the monograph.

The Community Pharmacy Report has a SWOT structure, as required by the University Directives. The placement took place in Aveiro, from April to August, where I learnt the work at a pharmacy, not only related with the deliveries and dispensing process, but also the management decisions and the structure of the pharmacy.

It can be found in the appendixes my report from the Hospital Pharmacy Placement, that happened under the Erasmus programme, in Salisbury, United Kingdom. The report follows the medication movement in the Hospital, as asked by the institution.

Key Words

Community Pharmacy, Orders, Pharmaceutical Care, Medical Prescription, Biochemical Parameters.

Capítulo 6

Introdução

De acordos com as normas do curso, efetuei um estágio curricular com mais de 648 horas em Farmácia Comunitária.

Escolhi a Farmácia Moderna em Aveiro, não só pela sua localização como também pelo seu grande número de lineares de cosméticos, e pelo bom atendimento que já me tinham proporcionado. O diretor técnico é o Dr. Fernando Bastos, e tão prontamente me recebeu na sua farmácia. Pude trabalhar com uma equipa constituída por mais duas estagiárias do 5º ano do Mestrado Integrado em Ciências Farmacêuticas, dois técnicos e duas farmacêuticas.

Comecei com a receção de encomendas, organização de lineares e gavetas e controlo de stock e validades. Treinei também simulações de atendimentos, em especial receitas médicas, para aprender a utilizar o software antes de começar a atender ao balcão. Comecei depois a fazer os primeiro atendimentos acompanhada sempre por um colega.

Em meados de Junho, o Dr. Fernando Bastos abordou-me no sentido de ir ajudar noutra farmácia da qual é igualmente proprietário, que se encontrava sem estagiários e tinha muito trabalho em mãos. Justificou que eu tinha um bom ritmo de trabalho, muitos conhecimentos, confiança no atendimento e boa postura em frente aos utentes e era a estagiária que melhor poderia ajudar na outra farmácia. Acedi ao pedido, e fui ajudar na Farmácia Nova, uma farmácia também no centro de Aveiro a 1km de distância, cujo diretor técnico é o Dr. João Bastos, filho do Dr. Fernando Bastos. A equipa tem ainda uma farmacêutica e quatro técnicas de farmácia.

Ambas as farmácias usam o mesmo software — 4D Care — o que não dificultou a adaptação ao novo local de trabalho, apesar de ter sido necessário passar novamente pelo processo de conhecer os espaços de uma nova farmácia. Inicialmente seria só uma ajuda temporária, e fui alguns dias isolados para a Farmácia Nova. No entanto, perguntaram-me se não me importava de continuar o estágio naquela farmácia porque contribuia ativamente para o bom funcionamento da farmácia, a equipa sentia-se confortável comigo a trabalhar lá e tinha já uma grande autonomia, o que permitia uma distribuição do trabalho na farmácia menos pesada. Concordei em ficar, e até ao final do estágio trabalhei maioritariamente ao balcão de atendimento.

Cada farmácia proporcionou-me um estágio diferente, o que me permitiu contactar com

uma realidade alargada, não só na diversidade de utentes, mas também diferentes métodos de trabalho e produtos vendidos.

O relatório do estágio prende-se por uma análise SWOT - Strenghts Wicknesses Opportunities and Threats (SWOT), onde exploro as Forças e Fraquezas que me são intrínsecas e influenciaram o meu estágio; e as Oportunidades e Ameaças, que me são extrínsecas, mas estão relacionadas com a farmácia em si e representam a forma como toda a farmácia, organização e equipa contribuíram para o meu estágio.

Capítulo 7

Análise SWOT

7.1 Forças

Conhecimentos prévios

Os conhecimentos adquiridos durante o curso permitiram-me ter uma abordagem crítica e adequada aos problemas dos utentes, bem como a detetar erros nas prescrições e na utilização de medicamentos ou dispositivos. Os conhecimentos sobre a organização da farmácia permitiram-me ajudar e opinar sobre a montagem de lineares tendo em conta as regras da rotação de stock e validades, e a aplicar os conceitos de *cross selling* e *up selling*. Para além disso, os conceitos dominados sobre parâmetros bioquímicos permitiram-me fazer uma análise crítica dos valores medidos na farmácia, e também em aconselhar medidas não farmacológicas e/ou farmacológicas aos utentes.

Aprendizagem rápida e entusiasta

Em farmácia há muito para aprender, não importa o quanto saiba do curso. Preocupei-me em me informar sobre as inovações farmacológicas, e sobre as antigas que ainda não conhecia, li muitos folhetos informativos e Resumo das caracteríticas do medicamento (RCM) de medicamentos, e folhetos das linhas de cosméticos. Assim, garanti sempre que conhecia os produtos que vendia.

Além de me ter empenhado em aprender sobre medicamentos de forma rápida e eficaz, também tive sempre interesse em aprender sobre a dinâmica da gestão da farmácia, perguntando e pedindo para participar em novas tarefas que fossem surgindo.

Organizada

A realidade da farmácia demonstrou-me que é necessário fazer diversas tarefas em simultâneo para se ser rentável. A minha capacidade de organização permitiu-me manter um registo de todos os telefonemas, encomendas e pedidos feitos, sem os confundir ou me esquecer.

Para além disso, uma das tarefas que fiz foi organizar prateleiras, gavetas e lineares. Ser organizada também permitiu fazer receção de encomendas de forma mais rápida e correta.

Facilidade de comunicação com os utentes e a equipa de trabalho

Conseguir perceber o que os utentes querem nem sempre é fácil, no entanto, fazendo as perguntas certas e conseguindo-me expressar, permiti que o utente ganhesse confiança em mim, e que o trabalho fosse feito com rigor. Apesar de no início ter tido alguma dificuldade em perceber as situações, rapidamente me tornei eficiente em comunicar com os utentes.

De igual forma, a equipa da farmácia sempre me ajudou e o facto de conseguir fazer-me entender e entender os outros profissionais, tornou o trabalho mais ágil, eficaz e fácil.

Domínio de línguas estrangeiras

Aveiro é um cidade universitária e turística, e como tal, tem muitas pessoas estrangeiras. Foram diversas os utentes que se dirigiram à farmácia que não falavam português, e o meu domínio de línguas estrangeiras como o inglês e o italiano, permitiram-me ouvir e comunicar com os doentes, e também ajudar os meus colegas da farmácia que não dominavam o inglês.

Domínio de técnicas de análise e monitorização de parâmetros bioquímicos e tensão arterial

Durante o curso tive a oportunidade de treinar a como medir a tensão arterial e de fazer punsões capilares. Estas técnicas foram muito úteis na farmácia, pois permitiram-me começar desde o início do estágio a acompanhar e monitorizar estes parâmetros de alguns utentes, acelerando o movimento da farmácia.

7.2 Fraquezas

Adaptação inicial a duas farmácias

A adaptação inicial à farmácia foi uma das minhas fraquezas tendo em conta que tive de decorar onde se arrumava cada categoria de medicamento ou produto da farmácia, a trabalhar com o software — 4D Care —, e a perceber o movimento pelo espaço. Como mudei de farmácia, este processo teve de ser repetido, tendo de me habituar novamente à localização dos produtos e aos espaços da farmácia.

Aconselhamento de OTC e MNSRM

Apesar de dominar bem os Medicamentos Sujeitos a Receita Médica (MSRM), o mesmo não aconteceu com os Medicamentos Não Sujeitos a Receita Médica (MNSRM) e *Over the count* (OTC). De facto, penso que os conhecimentos adquiridos no curso não são suficientes para

fazer aconselhamento aos utentes. Muitas vezes tive de pedir uma segunda opinião ou ajuda aos meus colegas para garantir que fazia um aconselhamento correto, e isso atrasou o atendimento. Houve vários temas que penso que consegui dominar no final do estágio, no entanto, ainda há muito que não sei sobre este tipo de aconselhamentos, que é cada vez mais necessário, mais complexo e exigente.

Tempo de atendimento

Uma vez que muita coisa era novidade e estava a exercer uma atividade de elevada responsabilidade, efetuei sempre os atendimentos com calma e confirmando todos os passos da venda do produto. Como tal, o meu tempo de atendimento era mais lento e isso atrasava a farmácia, o que por vezes incomodava os utentes que tinham pressa.

7.3 Oportunidades

Única estagiária

Quando comecei o estágio na Farmácia Moderna éramos três estagiárias, e repartir o espaço de estágio foi repartir a aprendizagem. No entanto, quando continuei o meu estágio na Farmácia Nova, era a única estagiária. Isso permitiu que conseguisse esclarecer todas as dúvidas, fazer mais tarefas e ter menos tempos mortos.

Ortopedia

A Farmácia Nova tem associada a si uma casa de ortopedia. Isso fez com que muitos clientes viessem à farmácia com prescrições de ortopedia ou com problemas que eram resolvidos com dispositivos ortopédicos. Os meus conhecimentos adquiridos na faculdade sobre ortopedia são muito poucos, e por isso, tive a oportunidade de conhecer uma vasta gama de dispositivos e sua utilização, falar com fornecedores novos e conhecer uma vasta gama de produtos.

Homeopatia

A Farmácia Moderna fazia encomendas de homeopatia todos os dias, e como tal, pude observar prescrições homeopáticas e conversar com os utentes que as faziam, alargando o meu conhecimento nestes produtos, nos seus efeitos, e a aconselhar e fazer vendas cruzadas com homeopatia.

Cosmética

A Farmácia Moderna e a Farmácia Nova tinham muitas marcas de cosmética, algumas delas diferentes de uma para a outra farmácia e, como tal, fiquei com um conhecimento muito alargado sobre as marcas do mercado e as suas gamas. A Farmácia Moderna tinha uma

especialista da área, com quem aprendi muito e me ajudou e ensinou a resolver os problemas dos utentes.

Dietética

A Farmácia Nova tinha também uma dietética. Alarguei imenso o meu conhecimento em produtos de emagrecimento e de fitoterapia, onde apliquei os meus conhecimentos adquiridos na universidade e fazia aconselhamentos tendo em mente a medicação do utente. Os meus conhecimentos permitiram-me detetar situações em que não era aconselhável determinado produto e ajudar o utente.

Para além disso, a Farmácia dispõem de um serviço de nutricionista, o que me permitiu observar o acompanhamento da mesma aos utentes.

Produtos Veterinários

Muitos utentes dirigiram-se à farmácia à procura de produtos veterinários. Pude contactar melhor com estes produtos, aconselhar e ajudar os utentes e os seus animais de estimação. A Farmácia Nova tem também uma parceria com o Hospital Veterinário de Aveiro, e por isso, surgiram muitas prescrições veterinárias.

Parâmetros Bioquímicos

A Farmácia Nova tem um gabinete onde é possível realizar a medição de vários parâmetros, nomeadamente medição da glicémia, colesterol total, perfil lipídico, ácido úrico, hemoglobina glicada e transaminases. Faz-se ainda análises à urina com equipamento que quantifica as tiras reagentes, o que permite detetar por exemplo potenciais infeções urinárias e assim aconselhar corretamente os utentes e fazer o encaminhamento médico quando necessário. Faz-se também testes de gravidez na urina.

Todos estes testes permitiram-me aplicar conhecimentos adquiridos, tanto ao nível da sua execução, como na interpretação dos resultados.

Formações

Foram inúmeras as formações que tive durante o estágio. Algumas foram durante o horário da farmácia, mas outras foram em horário extra-laboral e duravam algumas horas. Algumas das formações que tive foram sobre La Roche Posay® e Vichy®, Bioderma®, temática da menopausa da Gedeon Richter®, temática do tratamento da diarreia aguda da Merck®, temática da venda cruzada com Daflon® em insuficiência venosa, e alguns produtos da Jaba Recordati®.

Preparação de *blisters* de utentes

A Farmácia Nova e a Farmácia Moderna têm um protocolo com uma fundação de um lar de idosos, para a qual aviam e preparam a medicação, respetivamente. Na Farmácia Nova pude recolher e controlar as receitas dos utentes da fundação. Na Farmácia Moderna preparei os *blisters* semanais do idosos de acordo com as indicações médicas. No entanto, não se faz a revisão da terapêutica quando algum medicamento é alterado, ou surge um novo utente.

Administração de Injetáveis

As duas farmácias também fazem a administração de injetáveis, e por isso tive a oportunidade de observar e acompanhar tratamentos de alguns utentes, e fiquei mais elucidada sobre a administração intra-muscular e subcutânea no contexto prático.

Diversidade de tarefas

Enquanto estagiária, tive a oportunidade de fazer diversas tarefas na farmácia. Comecei pela receção e entrada de encomendas, a sua arrumação, reposição e monitorização. Fiz controlo e acerto de stock e validades. Atendi inúmeros telefonemas, resolvi problemas de fornecedores e de utentes, e fiz encomendas. Pude contactar com a organização das faturas, notas de crédito, pagamentos e integrações. Organizei lineares, prateleiras e preparei cartazes de promoções. Atendi ao público, aviando receitas e aconselhando, tirei dúvidas sobre a utilização de medicamentos e promovi a adesão à terapêutica. Contactei com eventos promocionais de marcas. Medi parâmetros vitais e bioquímicos.

Pude ainda fazer horas durante uma noite de serviço, contactando com a realidade que é passar a noite a trabalhar na farmácia.

Equipa

Ambas as equipas das duas farmácias me ajudaram muito, resolveram problemas comigo e tiraram dúvidas. Sempre bem dispostos, os meus colegas tornaram o meu estágio melhor, aliviando os meus momentos de dúvidas ou stress e encorajando-me a trabalhar cada vez melhor. Foram flexíveis com o meu horário e ajudaram-me com os transportes entre as farmácias, inclusive. Confiaram em mim e ensinaram-me tanto quanto puderam, mesmo em alturas de maior movimento da farmácia.

7.4 Ameaças

Medicamentos Manipulados

Nenhuma das farmácia faz medicamentos manipulados para os utentes, e como tal, não pude treinar ou observar as práticas. No entanto, faziam encomenda dos medicamentos

manipulados a outra farmácia, e por isso pude ver na mesma a prescrição de um manipulado e conhecer os regimes de comparticipação.

Nomes Comerciais

Como o ensino no curso foi sempre com os nomes dos princípios ativos e Denominação Comum Internacional (DCI), quando cheguei à farmácia, percebi que a realidade ainda é baseada em nomes comerciais. O facto de os utentes falarem e reconhecerem maioritariamente nomes comerciais não me permitiu ser tão eficiente numa etapa inicial.

Tempo de atendimento

A Farmácia Moderna tem um tipo de cliente mais calmo e com tempo. No entanto, eu fiz maioritariamente os atendimentos na Farmácia Nova que, sendo uma farmácia mais central e de passagem, recebia utentes que tinham pressa. A exigência na velocidade do atendimento não me permitia ser tão criteriosa, e forçava-me a ser muito rápida, o que me atrapalhava tendo em conta que não dominava tudo.

Farmacovigilância

Apesar de haver utentes a dirigirem-se à farmácia reportando reações adversas enquanto tomavam medicamentos, não tive oportunidade de ver os formulários e observar como se reportam estas reações na farmácia, e é cada vez mais importante na farmácia reconhecer e reportar reações adversas, garantindo a segurança dos utentes.

Tempo de pé

Praticamente todo o tempo de trabalho foi de pé. Não havia cadeiras altas nos balcões, era preciso subir a escadotes para ir buscar medicamentos e era requerida uma deslocação constante na farmácia. Apesar de ser só um estágio, tive de começar a usar meias de descanso e fiquei com alguns derrames venosos nas pernas. A exigência física dada às pernas é demasiada, não só para mim como estagiária, mas para todos os trabalhadores da farmácia, que resulta em problemas de saúde a longo prazo e condiciona a qualidade do trabalho.

Ausência de Robô na Farmácia

Como nenhuma das farmácias tinha robô, muito do tempo de estágio foi a arrumar as encomendas. Foi tempo que, embora me permitiu conhecer a localização dos produtos na farmácia e os nomes comerciais, me ocupava tempo de aprendizagem e de estudo dos medicamentos.

Capítulo 8

Conclusão

De uma forma geral, o estágio permitiu-me ter uma percepção do mundo da farmácia comunitária, do que o utente espera de nós enquanto profissionais, e das ferramentas que temos à disposição para ajudar.

Ter estado em duas farmácias deu-me a vantagem de perceber que cada farmácia tem as suas particularidades de funcionamento, mas que o foco é sempre o utente. Sinto que foi uma mais valia muito grande, não só por ter visto o meu trabalho e esforço reconhecido ao ter sido escolhida para ajduar na farmácia Nova, mas também por ter trabalhado com produtos diferentes que alargaram ainda mais o meu conhecimento.

Não só esta experiência serviu de ponte entre o conhecimento adquirido no curso e o trabalho profissional, mas também me ensinou a ouvir e comunicar com os utentes. A experiência foi muito enriquecedora, e sinto que progredi muito desde o primeiro dia de estágio, tanto por iniciativa própria ao me querer informar e gostar de saber mais, como também pela ajuda e conhecimento que os colegas da farmácia sempre me prestaram.

Ao longo do estágio, as minhas fraquezas foram desaparecendo, sendo que comecei a dominar os nomes comerciais e também a fazer um atendimento mais rápido e correto.

Os atendimentos ao balcão que fiz foram maioritariamente aviamento de prescrições médicas, sendo que fiquei muito treinada na abordagem a receitas manuais, materializadas e desmaterializadas, e a aplicar sistemas de comparticipação e despachos. No que toca ao aconselhamento farmacêutico, as temáticas mais frequentes que me surgiram foram infeções urinárias, candidíases ou vaginoses bacterianas, prurido cutâneo, queimaduras solares, diarreia, afonia ou dor de garganta e vista cansada.

Todas estas oportunidades contribuiram para que o meu estágio tenha sido muito produtivo, educativo e recompensador.

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Anexos

Polimer	Supplier	Origin	Preparation method	Particule Size	Surface and Shape	MW (kDa)	DD (%)	Viscosity	PDI	Zeta-potencial (mV)	Purification	Cells tested	Concentration	*in vivo* administration route	Antigen	Results	Controls	Ref	Date	Inflammasome			Cytokines														Cell Recruitment/activation							Ig				
																				NLRP3-/- mice did not induce IL-1β	phagocytosis is required	K+ efflux	IL-1β	IL-18	TNF-alfa	IFN-gama	IFN-β	IL-2	IL-10	IL-4	IL-6	IL-12	IL-13	IL-22	IL-17	Unprimed cytokines release	Neurophils	Eosinophils	Monocytes	Mast Cells	Macrophages	Lymphocytes	NK	Th1	Th2	Th17	IgG	IgG1	IgG2a	IgG2b	IgG2c	IgA	IgM	IgE
Chitosan	Primex	Shrimp	chitosan as bought	ni	ni	HMW	76 %	na	ni	ni	NaOH and heated at 90 °C for 1 h	BMDM	0.1 mg/ml	ni	ni	INFLAMMASOME. 3 h with 100 ng/ml LPS or left unprimed, and then stimulated for 6 h. Only chitosan primed induced IL-1β secretion.	unstim, chitin and alum	Chelsea L. Bueter et al. "Chitosan but Not Chitin Activates the In ammasome by a Mechanism Dependent upon Phagocytosis"	2011	X	X		X																													
			fractionated filtration	<100 µm [20 µm, 100 µm] <20 µm				na					1 mg/ml			INFLAMMASOME. priming with LPS 100 ng/ml for 3 h. IL-1β stimulation was higher with the smaller size	unstim, chitin and alum																																	
			dissolved in 10 mM acetic acid	soluble chitosan				ni					0.01 mg/ml			INFLAMMASOME. primed for 3 h with LPS 100 ng/ml. Low levels of IL-1β	Insoluble suspended chitosan																																	
			covered using acetic anhydride	beads with 3 µm and 50 µm				na					1 mg/ml			INFLAMMASOME. priming with LPS 100 ng/ml for 6 h. 3um beads coated with chitosan elicited a strong IL-1β response.	uncoated beads																																	
Chitosan	Primex - ChitoClear	Shrimp	solubilization, partial digestion with pepsin, precipitation, suspension and filtration	< 100 µm (pore filter)	ni	HMW	76 %	ni	ni	ni	0.1 M NaOH at 22 °C for 30 min	BMDM M1 Intermediate M2	0.1 mg/ml	ni	ni	INFLAMMASOME. primed for 3 h with 100 ng/ml LPS. 6h of incubation. Production of IL-1β more pronounced	unstim, chitin and silica.	Chelsea L. Bueter et al. "Spectrum and Mechanisms of In ammasome Activation by Chitosan"	2014	X		X	X	X	X	X																										
												BMDC				INFLAMMASOME. primed for 3 h with 100 ng/ml LPS. 6 hours of incubation. Production of IL-1β																																		
												peritoneal cells																																							
												human PBMCs																																							
Chitosan Salt	Novamatrix - Protasan CL213	ni	used as bought	ni	ni	150kDA-400kDa	75-90%	20-200 mPa.s	ni	ni	< 100EU/g	mice	1 mg	intraperitoneal	Hybrid antigen H1 (antigen 85B and from M. tuberculosis)	Th1-associated cytokine IFN-γ from CD4+ but not CD8+, limited production of Th2 cytokines or IL-17	na	Elizabeth C. Carroll et al. "The Vaccine Adjuvant Chitosan Promotes Cellular Immu- nity via DNA Sensor cGAS-STING-Dependent Induction of Type I Interferons"	2016		X		X				X	X				-													X		X				
												Ifnar1−/−, draining mediastinal lymph node				Decreased capability to induce IFN-γ and IgG2c	na																															
												BMDC and BMDC withour TLR4	ni	ni	ni	enhanced surface expression of CD40 and CD86 by DC, no secretion of IL-12 or IL-6	positive controls: LPS and CpG																																	
												DC				inducing the expression of both Ifnb and Ifna mRNA, induction of CXCL10, an ISG.	positive controls: LPS and DNA																																	
												DCs from Ifnar1−/− mice				IFNAR signaling contributed to a feed-forward loop of IFN-β, no Ifna mRNA transcription, low upregulation of CD40 and CD86	positive controls: LPS and CpG																																	
												DC from WT and STING-deficient				Induction of Ifnb, DC maturation, secretion of IFN-β and CXCL10 in response to chitosan was completely abolished in Tmem173−/− DC	LPS																																	
												DCs from cGAS-deficient mice				no induction of Ifnb mRNA and Cxcl10 expression; secretion of IFN-β and CXCL10 abolished	LPS																																	
												CD4+ cells from PEC from Tmem173−/− or Mb21d1−/−	1 mg	intraperitoneal	H1	IFN-γ response and IgG2c reduced	na																															
												restimulated lung cells	50 µg	intranasal	PspA	specific IFN-γ production	na																															
CNP	ni	ni	olive oil in their hydrophobic core and epikuron on their shell	340 nm	spherical	ni	ni	ni	< 0.15	ni	ni	PBMC - T cells, B cells, monocytes, dendritic cells and natural killer	1 × 10^11 mL−1	na	na	Tcells (CD3+) and Monocytes (CD14+) uptake CNP. Induced cell apoptosis in T lymphocytes and monocytes. Increased monocyte diameter. Powerful CD25 and CD69 modulation on both immune cell types.	untreated cells	Cristiano Farace et al. "Immune cell impact of three di erently coated lipid nanocap- sules: pluronic, chitosan and polyethylene glycol"	2016					X	X		-	X	X	X	X	X												X	X						
CNP	Sigma Aldrich	ni	ionotropic gelation	739nm	rough surface	LMW	ni	na	0.266	ni	Filtration through a 0.22µm pore	Mice BMDC	50 mg/ml for 24 hours	na	na	INFLAMMASOME. CNP were able to induce IL-1β secretion, with previous prime with LPS 50 ng/ml for 3 hours.	lps, bpn, ifa, alum, cub	Silke Neumann et al. "Activation of the NLRP3 in ammasome is not a feature of all particulate vaccine adjuvants"	2014				X		X																										
												murine peritoneal macrophages colected from peritonial cavity		na		INFLAMMASOME. CNP were able to induce IL-1β secretion (colected 4 hours after)																																	
												Human monocytes		na		INFLAMMASOME. CNP were able to induce IL-1β secretion, with previous prime with LPS 50 ng/ml for 3 hours.																																	
												macrophages peritoneal cavity	3 ug	intraperitoneally injection		INFLAMMASOME. CNP were able to induce IL-1β secretion (colected 4 hours after)																																	
Chitosan Salt	Novamatrix - Protasan CL 213	ni	used as bought	ni	ni	150kDA-400kDa	75-90%	20-200 mPa.s	ni	ni	<100 Eu/g	BM imature DC	0.016–2 µg/mL	na	na	INFLAMMASOME. Stimulation with LPS (5 ng/mL), after 24 hours chitosan promoted optimal IL-1β and did not inhibit IL-12. Primed with CpG 4 µg/mL and mesuared after 24h induced IL-1β secretion. did not induce significant antigen-specific IFN-γ or IL-17 responses in the mediastinal lymph nodes or spleen but these adjuvants did promote an antigen-specific IFN-γ response in PECs.	PLG and unstim	Andres Mori et al. "The vaccine adjuvant alum inhibits IL-12 by promoting PI3 kinase signaling while chitosan does not inhibit IL-12 and enhances Th1 and Th17 responses"	2012	X	X	X			X		X																									
												PEC mediastinal lymph nodes or spleen blood serum	1 mg	intraperitoneal immunization subcutaneously	ovalbumin (50ug)	antigen- specific IFN-γ and IL-17 did not induce significant antigen-specific IFN-γ or IL-17 responses anti-OVA IgG1, IgG2a and IgG2b enhancement	PBS, OVA, alum																																	
CNP	Chitosan Company of Pan'an, Zhejiang, China	ni	coacervation between postiv. charged chitosan and negat. charged sodium tripolyphosphate	83.66 nm	regularly formed	220 kDa	95%	na	<1	35.43 mV	0.22 µm Millipore filter and autoclaved. <0.5 EU/mL	Spleenocytes from mice	12.5, 50, 200 µg	subcutaneously	OVAlbumin (25ug)	CNP could promote lytic activity of NK, splenocyte proliferation, IFN-γ, IL-2 and IL-10 (Th1 and Th2)	ovalbumin, a saline control, and a positive control (QuilA)	Zheng-Shun Wen et al. "Chitosan nanoparticles act as an adjuvant to promote both Th1 and Th2 immune responses induced by ovalbumin in mice"	2011						X		X	X																									
CNP	Chengdu Organic Chemistry Institute of	ni	ni	45 nm	ni	ni	ni	na	0.190	25.6 mV	ni	blood from tail vein	5 pmol	intramuscularly oral	na	No difference between the oral and intramuscular levels	saline and CpG alone	Kai-Yuan Wu et al. "A novel chitosan CpG nanoparticle	2006									-		-	-			-			X					X		-	-					
Chitosan Salt	NovaMatrix - Protasan G213	ni	ni	ni	ni	200-600 kDa	75-90%	20-200 mPa.s	ni	ni	<100 EU/g	mice spleen, CD4+ Blood Serum inguinal lymph nodes and spleen	1.5% chitosan dissolved in PBS	subcutaneously	β-galactosidase (100 µg)	proliferation was higher than saline and equal to IFA and IgG induced (IgG1 and IgG2a) significant increase in the size of the lymph nodes, they were dissected and showed the number of leukocytes and NK increased	ovalbumin (negative control), concanavalin A(positive	David A. Zaharo et al. "Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous	2007																X	X	X	X		X	X										
Chitosan solution	Sigma-Aldrich		0 5% chitosan solution was prepared by dispersing it in acetic acid and then add 40 mL distilled water	na	na	ni	ni	ni	ni	na	121°C for 15 min before using	immunocompetent and immunosuppressed rats lungs	0,3 mL of 0,5% chitosan solution	tail vein injection	na	CD4+ T-lymphocyte increased and CD8+ decreased. Reduction in the parasite mRNA level.	model and acetic acid	A.-B. Liu et al. "Therapeutic e cacies of chitosan against Pneumocystis pneumonia of immunosuppressed rat"	2014						-	X		X								-	X															
CNP	Primex BioChemicals AS (Avaldsnes, Norway)		Na2SO4 to a CS solution under magnetic stirring	396.2 ± 35.0 nm	ni	LMW	95%	na	0.156	21.59 ± 2.81 mV	purification process	Human mast cell	20, 40 or 80 ug/mL	na	na	induced mast cell activation	C48/80, Chi-C48/80	D. Bento et al. "Development of a novel adjuvanted nasal vaccine: C48/80 associated with chitosan nanoparticles as a path to enhance mucosal immunity"	2015						X		X	X	X			X	X			X									X	X		-				
												Blood samples				Detected IgG																															
												Mice Vaginal wash	50 ug/mL	intranasally	PA (2.5 ug)	IgA not detected																															
												Fecal Pellets				IgA Detected																															
												Nasal lavage				IgA not detected																															
Chitosan solution	Primex Bio- Chemicals AS (Avaldsnes, Norway)		acidified with acetic acid	na	na	LMW	95%	8 cP	na	na	0.22 µm filter	mice cell spleenocytes	0.25% (w/v), 0.125% (v/v), and 0.01% (v/v)	na	na	an enlargement in both forward and side scatter, and cellular granularity. It affected CD4^+, CD8^+ and B cells. expression of CD69 but did not proliferate	CpG ODN, Con A and negative control	Olga Borges et al. "Induction of lymphocytes activated marker CD69 following exposure to chitosan and alginate biopolymers"	2007																X																
CNP and microparticles	Sigma- Aldrich		emulsion with stiring and cross-linking	113–213 nm and 1.62–2.58 µm	ni	164 kDa	75–85%	0.026 Pa s	ni	ni	ni	BMDC	25 and 50 µg	na	LHRH	uptake ocurred occurred in a concentration- dependent manner	PBS and protein in saline	Brendon Y. Chua et al. "Chitosan Microparticles and Nanoparticles as Biocompatible Delivery Vehicles for Peptide and Protein-Based Immunocontraceptive Vaccines"	2012																																	
												popliteal lymph nodes	200 µg	footpad		DC carried the particles to the draining lymph nodes, CNP were faster																																	
												sera	particles containing the equivalent of 25 µg of protein	subcutaneously at the base of the tail		antibodies IgG were detected																															
Chitosan suspension	Fionac C, Kyowa Tecnos Co., Ltd., Japan	crab	chitosan flakes pulverized.	3 µm	ni	80kDa	82%	ni	ni	ni	ethylene oxide gas	human serum	1 ml of 0,1, 1, or 10 mg/ml	na	na	COMPLEMENT: C3 and C5 decreased while C4 did not change. Alternative pathway	zymosan, physiological saline, serum	Saburo Minami et al. "Chitin and chitosan activate complement via the alternative pathway"	1998																																	
Chitosan solution	Sigma Ltd. (St. Louis, MO, USA)		soluble with sonication in an aqueous solution of 4% acetic acid	371 nm	round shape	71.3 kDa	80%	2.54 cP	ni		0.22 um filter	Chicken blood	50 ml of 0.5 mg/ml, 1.0 mg/ml, and 2.0 mg/ml	orally	na	did not induced IgA	saline.	Jing Zhao et al. "Cell outer membrane mimetic chitosan nanoparticles: preparation, characterization and cytotoxicity"	2015																																-		
CNP	TaeHoon Bio. Co (Korea)		acetic acid and sodium tripolyphosphate with stirring	220 ± 23 nm		33 kDa	90%	2.8 cP	ni	22.7mV	ni	BAL fluid from mice lungs	4 mg/mL	intranasal	ovalbumin 50 µg	no reduction of eosinophils nor induced apoptosis in inflammatory cells	saline and untreated mice	Dong-Won Lee et al. "Thiolated chitosan nanoparticles enhance anti-in ammatory e ects of intranasally delivered theophylline"	2006															-																		

Salisbury NHS Pharmacy Placement Report

Helena Ferreira Lopes da Silva Santos

9/01/2017 - 7/04/2017

Contents

Chapter 1

Introduction

My placement took place at *Salisbury NHS* for three months, where I spent seven weeks in Stores and six weeks in Dispensary and Aseptics. From time to time, I went also to the wards.

With this placement, I got the opportunity to understand and have an active contribution in the flow of the medication at the hospital, that now I write on this report.

As it can be seen in Figure 1.1, briefly, a drug is requested, ordered, registered when arrives, stored, dispensed and can also be returned or disposed. This flowchart will be the starting point of the report and all the items there will be analysed and explained, more or less, according to my role in each.

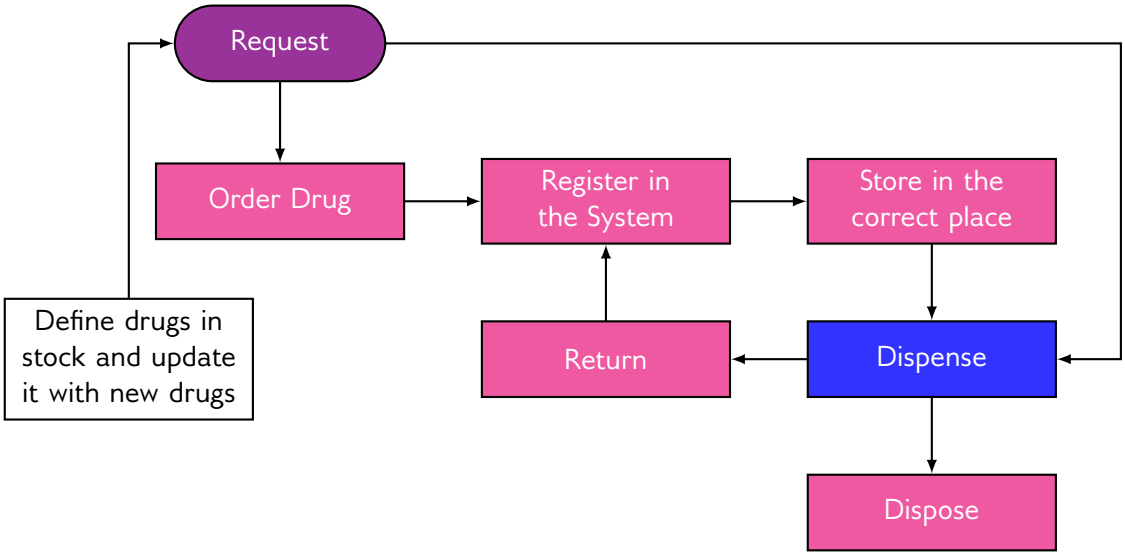


Figure 1.1: The medication cycle in the Pharmacy. The colour Pink indicates that happens in Stores, the colour Blue indicates that happens in dispensary, and the colour Purple indicates that happen in both.

I am going to start with Stores and talk about a little bit of everyone's role, and more deeply about the returns and stock check, where I spent most of my time and contributed in a positive and innovative way. Then, I am going to describe my experience in Dispensary and the exceptions to pay attention when dispensing a medication.

Chapter 2

Stores

Stores is the area of the Pharmacy were medication packages are received, introduced into the system, stored and assembled so they can be delivered to the whole hospital and patients. This area is managed similarly to a warehouse, and the main concerns and focus are the correct store and protection of the drugs, the easy access to them by the workers of the pharmacy and the maintaining of the stock levels.

The persons responsible for this are pharmacy assistants, pharmacy technicians, and a senior pharmacy technician — the person with the highest responsibility in stores. All of them have different responsibilities and work together so that the flow of the drug is not interrupted.

Everyday there is a particular routine that assures the correct operation of Stores, that is going to be described with more detail later. Briefly, a drug is ordered, arrives, is introduced in the system, is stored properly, and is delivered to the destination, that can be a patient or an ward.

Furthermore, there is also administrative work that includes auditing, stock check, invoices issues, expiry date control, but not all of them are not going to be discussed so thoroughly since I did not have a major contribution in many of these fields.

The Pharmacy stock control system is JAC, and allow the user, if it has the right clearance, to give entrance of new drugs in the system, book out a drug, search its location, return a drug, analyse TF, and much more. Everybody has credentials to access the system, but they have different levels of clearance which controls what they can see or do in JAC.

In resume, Stores is the key to a correct, fast and proper drug supply, and the way they work defines the progression of the Hospital itself.

2.1 Receipt of Deliveries

The first step for every drug is its delivery. Each delivery must be accurately checked against the delivery document in order to clear any possible discrepancies. The discrepancies can be of varied nature:

- wrong delivery address

- different item
- pack size
- quantity
- expired or short date stock

When everything is checked and is correct, the items are booked in via JAC. Once they are in the system, they can be stored and dispensed. This is all resumed in Figure 2.1, as a flowchart.

There are special precautions with fridge items, since they need to be booked immediately after they arrive, so they can be store in the fridge before deterioration. Another precaution is needed when handling or storing CD, chemotherapy drugs and specials or unlicensed stock, since they have a more strict control.

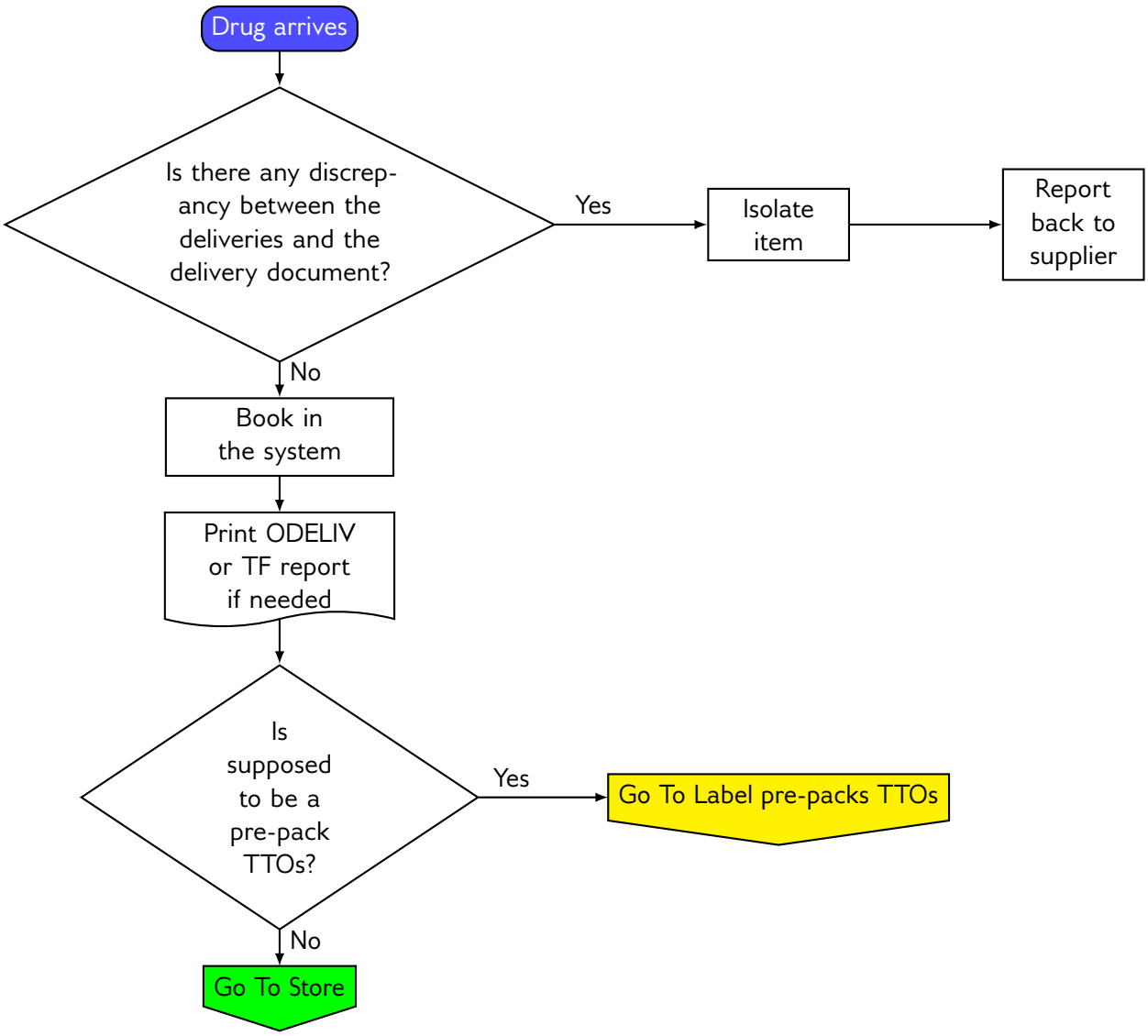


Figure 2.1: The drug arrival procedure.

2.2 Storing Stock

When the deliveries are booked in as stock, they must be stored, like it is represented in Figure 2.2. Many of the items are stored inside the robot. There are two options to feed the robot – the boxes can be placed in the hopper and the robot store them itself, or sometimes the robot can not do that, and they must be stored manually on the robot. There are many reasons for the robot not store packages itself, like:

- braille writing
- too heavy
- too shiny

And so, the robot has to be fed.

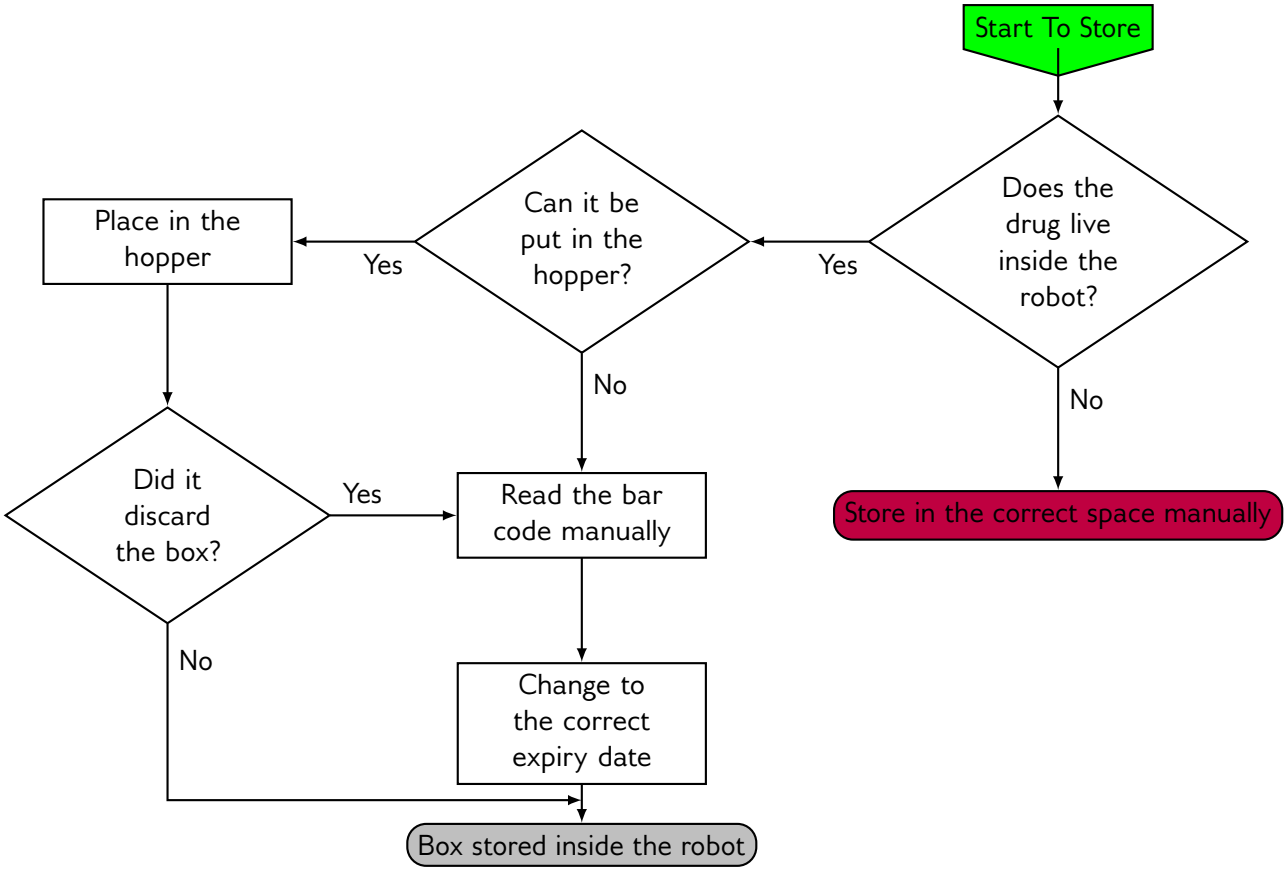


Figure 2.2: The storing procedure and feeding the robot.

When the packages are stored manually in the robot, if they have QR code, it must be read with priority, because it includes the correct expiry date already. Sometimes, packages are not kept inside the robot. They have/are:

- strange shapes
- too heavy
- too fragile
- too large

- unreadable bar code

and because of that, they have to be stored in Stores, Dispensary, Shop, EDC or others. Currently, Stores has shelves to dressing products, intravascular (IV) bags, injectables, "magic island" – for products without a specific category –, family planing, food and pre-labelled TTO packs.

In dispensary are stored creams and ointments, liquids and the DU's Qty.

Shop has low quantity of stock and the drugs are all over the counter (OTC) and they represent the top selling products.

All stock places follow straight rules related with Health and Safety, and stock rotation. Stores has temperature monitoring, and so have the fridges.

2.3 Ward Distribution

The medication can be given to a patient or distributed to each ward. With ward distribution, the Pharmacy ensures that the medication can be provided to the patients, when in need, by the nurses, in a much faster and effective way. The drugs are kept inside the treatment room, looked away from the patients.

2.3.1 Top-up

The items and their quantities are decided according to the needs of the ward — for example, the burns ward needs more dressing items then the paediatric ward which on the other side, needs more liquid medication then the burns. The personnel from Pharmacy are responsible to do top-up service once or twice a week in order to monitor the amount of each drug available and its expiry date.

All the items are checked against the bulk issue profile, where it is written the minimum amount for each product. If the number of boxes is lower then the minimal quantity, it should be written how many are needed in order to fill it up.

Once the bulk issue profile is completed, it must be delivered to Stores so that the missing drugs can be replaced. The Figure 2.3 resumes this process.

2.3.2 Picking boxes

Picking boxes is the direct consequence of the Top-ups. Once the bulk issue sheet arrives, the bar codes that correspond to missing drugs are read, and a pick box with a list of the drugs needed and the amount for each is created (2.4).

As many of the drugs are stored in the robot, is also the robot the one how picks the those boxes, and deliver them to a green box through a belt. The items delivered by the robot must be checked, since it can send different quantities of those requested.

When the double check is done, all the items that do not live inside the robot must be picked manually from the shelves. It is not common to have items on the picking list requested

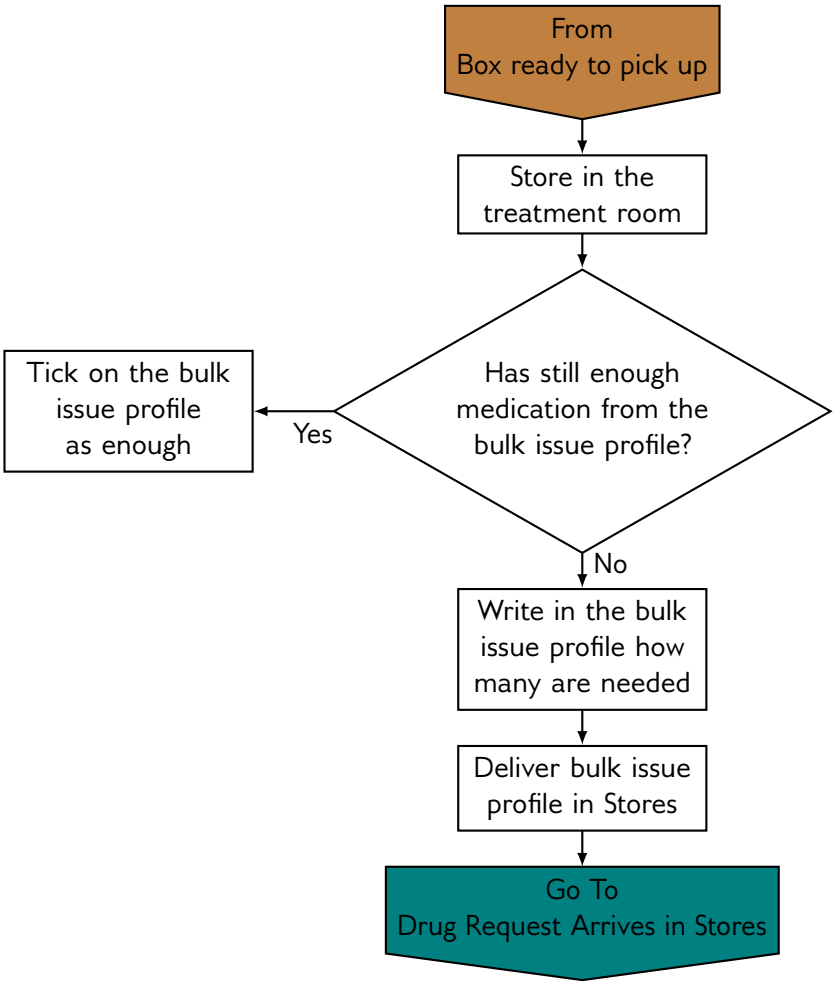


Figure 2.3: The Top Up in the wards

that are not in stock, since the system does that automatically, but since there can be stock disparities, if that happens, it must be written that the item in question is a TF.

The boxes must be double checked, and then sealed and displaced is the hall to be picked by the porter.

There is a cycle between top-up and boxes picking, and they are usually made in the morning.

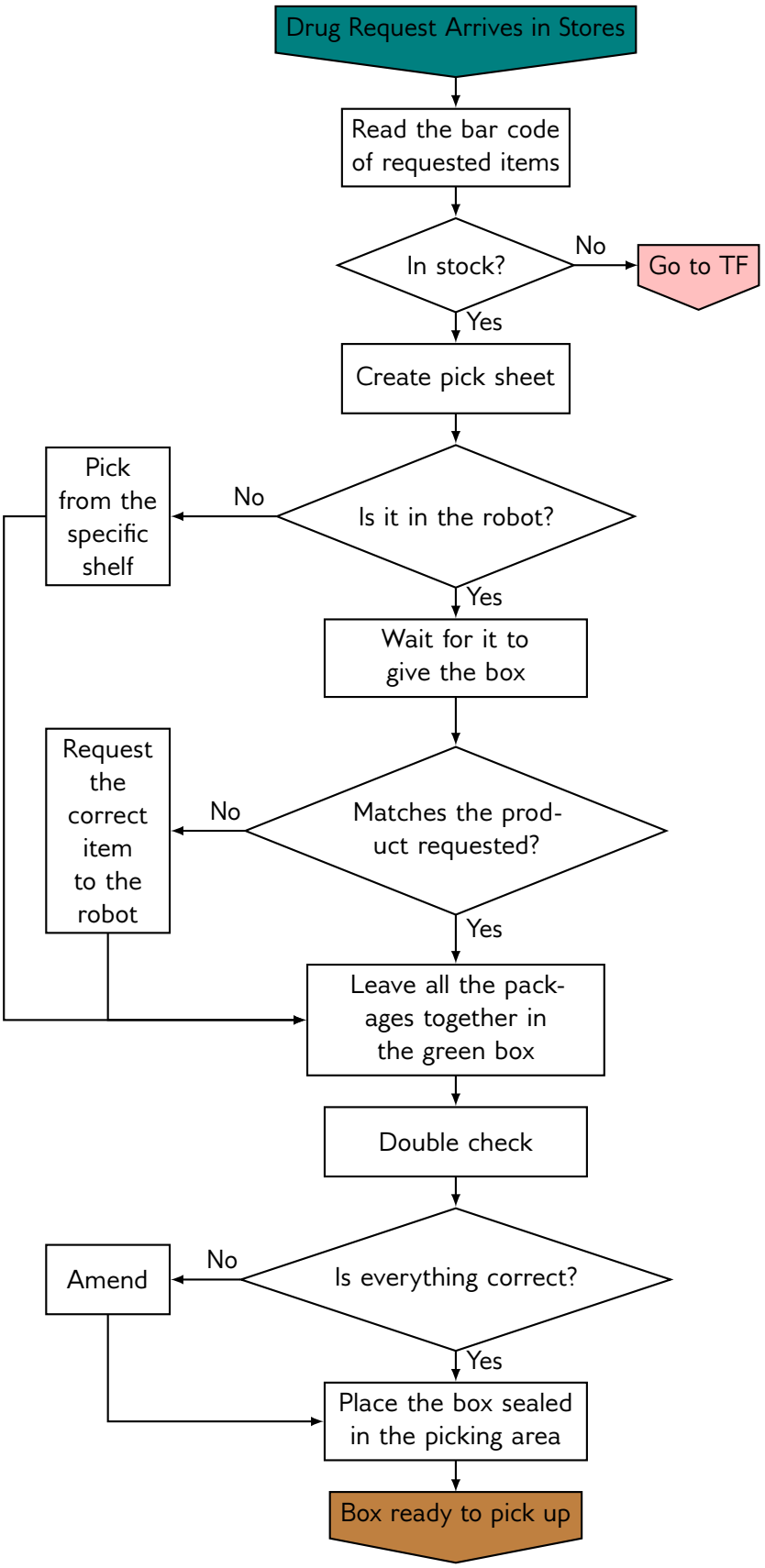


Figure 2.4: The boxes picking procedure.

2.4 TF

Sometimes, it happens that Stores does not have the medication needed. It that case, a TF process is created on JAC, so that in the next delivery, if the drug of interest arrives, it warns the person and the box is put aside to be delivery to the ward or patient who is waiting for it.

If it is an emergency and the drug is needed with brevity, it can be made a special order from another deliver company, that might not be the one which the hospital has contract with, but it will arrive quickly — Figure 2.5).

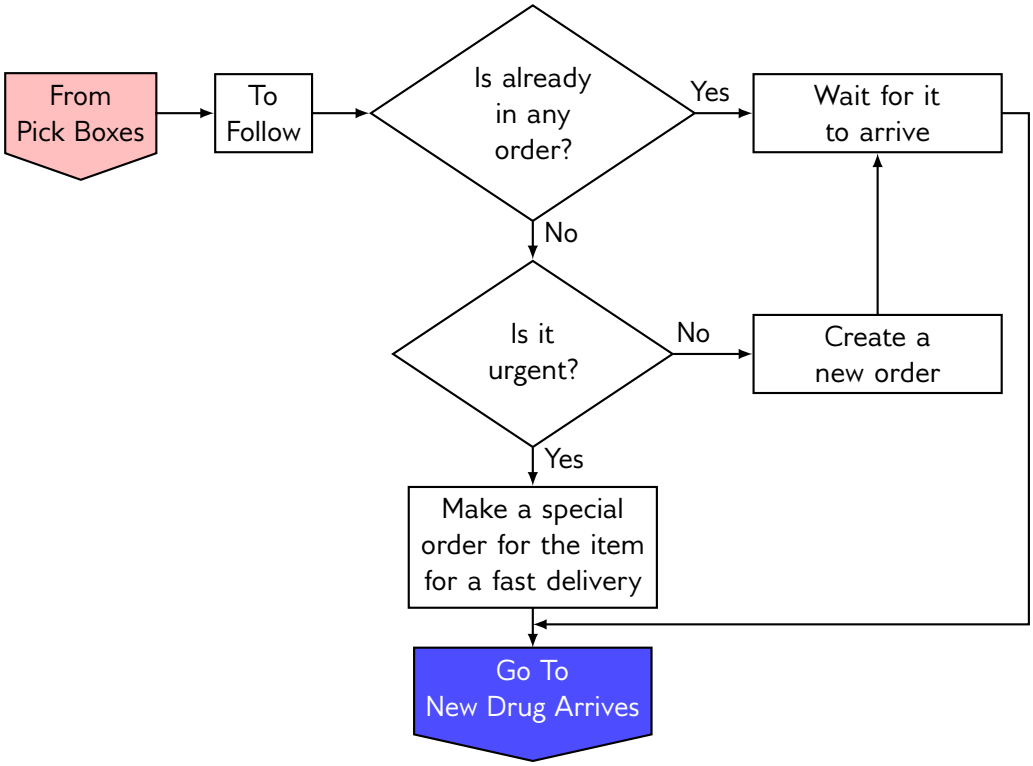


Figure 2.5: The TF process.

2.5 Labelled pre-packs TTO

Although dispensary has the main job to dispense drugs with personalized labels to each patient, some wards can have pre-labelled packs where they can write the personal information of the patient, and, with that, speed up the discharge process, since it can be slow when the patient has to wait for the drugs supplied by the Pharmacy.

There is an established list of drugs that are pre-packs TTOs, and the respective labels are already defined and ready to print. Some of the pre-packs are labelled by the industry who manufacture the drug.

When one of those drugs arrive, the labels are printed - if they are not already labelled - and they must be stuck on the boxes in a way that they do not hide important information written in the cardboard. Some boxes have already space to write the patients name — the paracetamol boxes, for example — and in that case, only the hospital label is required.

It is also of high importance to add special information labels when required — for example, contains penicillin warning —, so that the patient can be alert to any reaction/side effect when taking the medication (2.6).

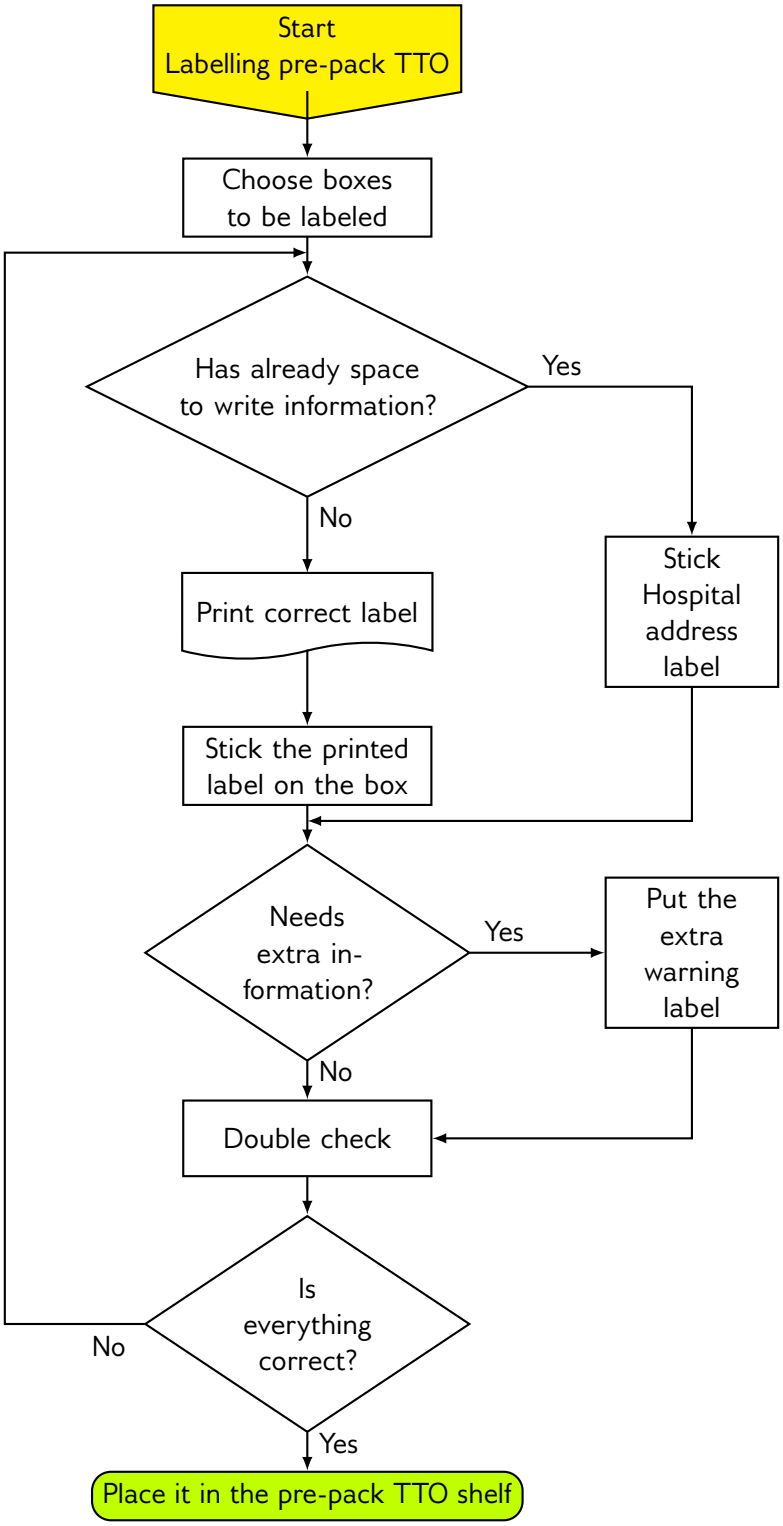


Figure 2.6: The pre-packs TTOs labelling process.

2.6 Returns

It is policy of the hospital to restock some of the dispensed drugs. It is usual that, when a medication is no longer needed in the ward or by the patient, it is send back to the Pharmacy in order to be disposed or returned. This is a delicate procedure since Pharmacy can not book in items that can be hazardous or are contaminated in some way.

The main rule is that if it is not from the hospital or if it got out of the hospital, the Pharmacy can not assure the integrity of the drug and it must be disposed.

Secondly, if it is out of date, it has be disposed.

There are rules to dispose and they can be found in more detail in flowchart 2.7. It Is to emphasize that every time a drug is placed in the yellow bin – for the hazardous drugs – it has to be written on a sheet the drug disposed there, why was it disposed, and the quantity.

There are a huge variety of drugs that can arrive back to Pharmacy.

Patient own drugs (POD)

The PODs are the items that are property of the patient and were brought to the hospital by the patient when he was admitted, but are no longer needed. They must be disposed since they came from outside the hospital.

Ward items

Wards can also send back some items from their own cupboards that they no longer need or are out of date. They do not have an hospital label and because of that, if in good conditions, they must be booked in immediately so that the next person doing the returns does not wonder where they come from and dispose good items.

Inpatient medication

These are the boxes that are dispensed by the hospital when the patient is there, and are given back because he no longer needs them. In this case, they can be booked in again if they are not:

- damaged
- close to expiry date
- fridge item with unknown duration outside of the fridge
- loose tablets/capsules or with unknown expiry date
- broken seal — liquids, ointments, creams, gels

2.6.1 Returning products to Pharmacy Stock

When the items are sorted, they can be booked in again in the system. They must match the patient or ward from where they come from and the DU's Qty ou packages must be written.

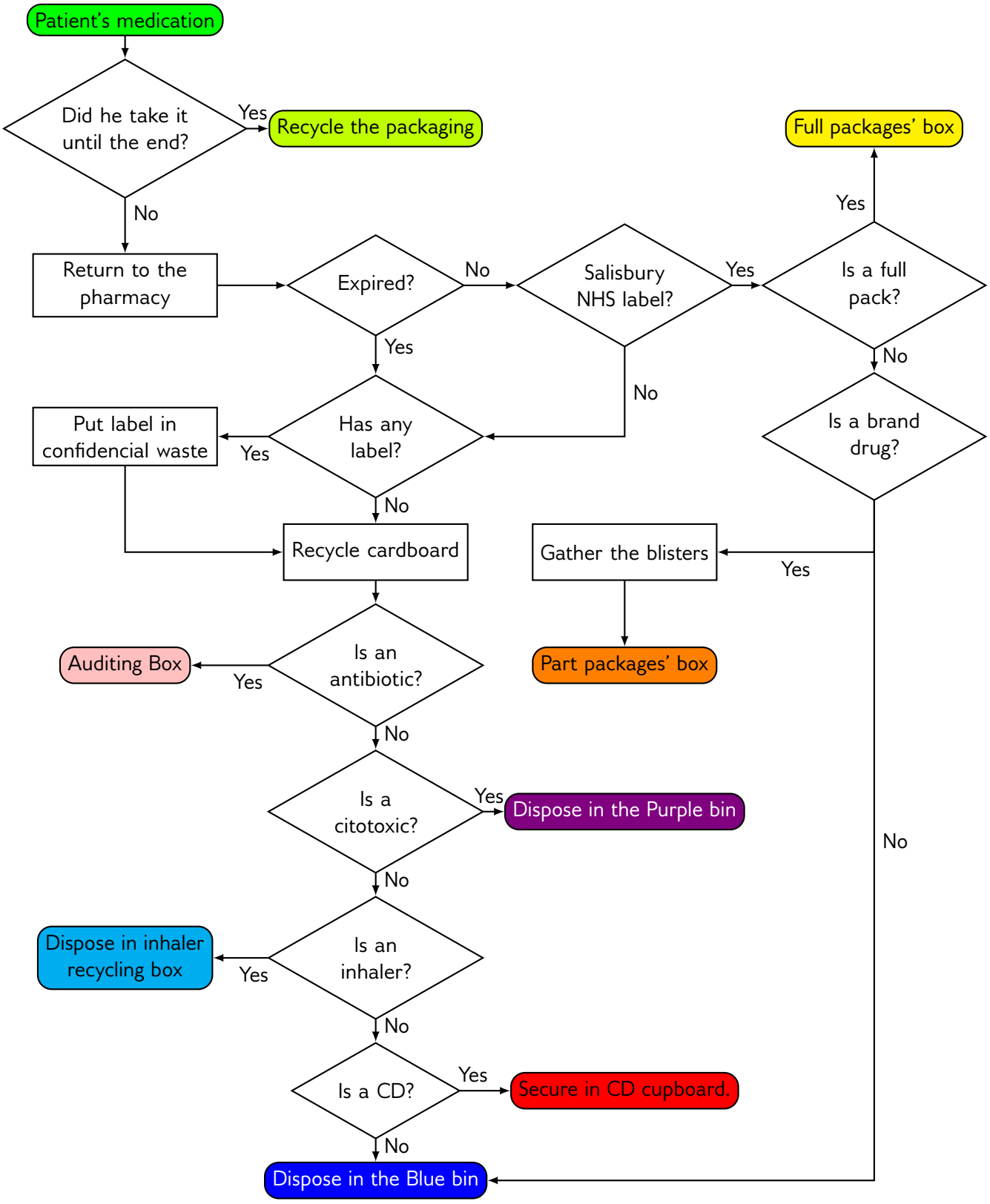


Figure 2.7: How to sort the returns

Once in the system, the part packs can be stored in Dispensary, and the full packs can be stored in their original place of living – robot, shelves, dispensary, shop...

2.6.2 My role in the returns

I spent a lot of my placement doing returns and with that I came across with some problems, one that I amend but others that require more time and are written here for the future.

The "Sorting Medication" Box

A problem that I realized from the beginning was that when a bag was brought back from the wards to be sorted, it would be left on the floor which not only looks bad to someone new who arrives to Pharmacy, but also it is conducive to the lost of drugs and poor conditions for storing. Worst was it when drugs not checked were placed inside the boxes for sorted items, mixing the wrong ones with the ones ready to be introduced in the JAC. That made me spend more time doing returns because there were always boxes on the wrong place.

With this scenario, I decided to transform a black box that was no being used – the one for the liquids and bottles – in to the "Sorting Medication" Box, where all the drug and bags are kept before being sorted. This was well accepted by the pharmacy employees and it greatly reduced the errors. This changed made easier the sorting of the medication and also kept the corridor clean and easy to walk through.

The untrained personnel

For some reason, some people do not give them time to learn how to do the returns and they do it wrong. With good intentions, I am sure, some people sort drugs to be returned that can not be returned. And this is a big problem, because the person that is doing the return on JAC might make a mistake and this power rate big problems like returning out of date drugs, medication that is not from the hospital or even book in a drug that does not match the box.

It is also a big problem in the returns when people do not dispose the drug in the correct bin. I found hazardous drugs on the common blue bin, simple drugs on the yellow bin, or labels with personal information on the cardboard recycle box.

Outpatients returns

The problem I think is the major one is book in outpatient drugs. A drug that left the hospital can not be returned — we no longer can assure that it was kept with the correct conditions. And so, we rely on the hospital label to recognise those situations. But what happen to the outpatients? Their label is exactly like any other label from an inpatient but left the hospital, so it can not be returned. The only way to know if it is an outpatient is when we are already returning on JAC and we insert the code that identifies the dispensing. After that, the category of the patient shows up discretely. We could, on that point, dispose the drug and not return it if it says "outpatient". But a new question shows up. Can we rely on that information, or could have the person that labelled selected the wrong cathegory for the patient? It is highly important for the labellers to be ware of this situation, because if, for any reason, they categorize the patient as an inpatient when is an outpatient, we are returning medication

that might not be already usable, or if the opposite happens, the hospital is losing drugs and money that could be returned.

I suggest that besides this, a mark/symbol/reference is added to the label or by the dispenser on the box for every drug dispensed for an outpatient. With this, even if the labeller gets the cathegory wrong, there is an easy mark for the person responsible for the returns to identify it easily, and also dispose it during the screening and not only when is already at the computer.

The same question can be extended to a patient that leaves the hospital and by an unfortunate event gets back with that same medication and it is returned. We can not return that, we do not know if is safe. Probably everything is normal with the drug, but if the hospital gives something that is spoiled and a patient dies because of that, for example, is it major problem. Returns are very serious and there are hidden risks that people are taking very light.

For this, I suggest that the pharmacist/Medicines Management Technician (MMT)/nurse responsible to give the medication to the patient when he is discharged mark it the same way as the boxes for outpatients, or to be already written on the labels when it is printed for a TTO.

Pre-pack TTO

All the drugs that are pre-packs should not be returned because we can not assure their integrity, even with the hospital label. If it comes from the ward, it should be written that information so that the person responsible for the return on the computer knows that it is safe to return.

Unlabelled boxes

Sometimes, some unlabelled boxes are on the medication to sort. They can come from a patient, but can come also from the ward, and that means that they never left the hospital and can be returned. A note should be written on the box so the person responsible for the returns knows that is safe to return, specially if it is an expensive drug. That would save more money to the hospital because more safe products were being returned.

Bottles

I personally think that any tablet or capsule that is on a bottle should not be returned if:

- it was already opened
- does not have a security seal
- it was changed from the original package to a dispensary bottle.

This is because we do not know if the patient was with clean hands when he took a pill, or if he took all of them off to a different container and put it back, or swapped drugs, or even more nasty situations. For that, all of them should not be kept.

Conclusion on the returns

This actions would cover the majority of hidden risks, would reduce heavily the situations that could put a patient in danger and would save money to the hospital. If we have such careful in Dispensary to give the right drug to a patient, we must focus first on the drug we are picking from the shelves.

2.7 Ordering

Ordering is the only way to get a drug from a supplier. It should be done everyday, and it is as task that requires a prospective thought, because we never know what can be needed on the next day. It takes in consideration the stock available, the orders from consultants and the average stock booked out from the previous months. There are two big groups of orders — the ones that the system generate by itself every time a product goes below the minimum quantity established as stock, and every time a person requests a drug that is missing in stock. After this, all orders have to be released by the manager of Stores, who decides ultimately if that item needs to be order or not.

2.7.1 Contract Maintenance

My contribution in ordering was to update the contract information on JAC. The NHS group has different contracts that are used by all the hospitals, but not all the products that are in the contract are chosen to be in stock. The contracts allow the Pharmacy to buy the products cheaper comparatively to a purchase without the contract. My job was to add the new contracts to each supplier, adding the main information:

- Contract Number
- Effective start date
- Effective end date
- Honouring suppliers for contract
- Drug
- Price

By updating the contracts, every time now a drug is out of stock from the first supplier, it is easy to see who is the next supplier, and, with that, order the item only from contract suppliers, saving money to the hospital.

2.8 Stock Control

As said before, not always the real stock matches the stock written on JAC. So, to correct that, a physical stock check must be made frequently. along with that, the expiry dates must be also checked so that any product is never dispensed out of date.

2.8.1 Robot stock check

In Robot stock check, every night the robot prints a letter — the KROB list — comparing the amount of each drug inside it and the amount of items that JAC has register as booked in. This must be analysed when the hopper is empty, and the discrepancies must be investigated.

There are many reasons for this discrepancies, like with items that:

- do not live in the robot
- were take out of the robot for being out of date but were not still booked out of the system
- live in the robot and also in dispensary/shop
- were in the hopper when the check was made
- are missing

I had a major contribution in this particular topic, since I created spreadsheets to analyse and filter the information in the KROB letter, so the biggest issues would pop out and we could isolate them and solve them. More information about the spreadsheets can be found in appendix A.

2.9 Other side tasks

Until now, I wrote about the cycle of the drug inside the store and it's control. But there are others side tasks equally important where I got involved.

2.9.1 Emergency boxes

The emergency boxes are special boxes that are kept on the wards with particular medication that is needed in case of any emergency. There are three types of boxes:

- cardiac arrest
- anaphylaxis drugs
- paediatric

Inside each box is kept emergency drugs, and they are assembled by the personnel from Stores. We guaranty that the drug inside the box has a far expiry date, and we keep records of the batch number of each product inside de box.

Once they are completed and doubled check, they are sealed, given an expiry date that match the shortest expiry date inside, and they are kept on the wards. When the expiry date is near or the box is used, it returns to Stores so they can be assembled again.

2.9.2 Clean green boxes

The green boxes are the boxes used to carry the drugs to the wards. Therefore, they are exposed to a potential contaminated environment, and can become fomites for different pathogenic diseases. When they arrive back from the wards, they are disinfected with chlorhexidine wipes, clinical tested again microorganisms, so the speed of diseases can be contained.

Chapter 3

Dispensary

In dispensary, contrary to Stores, each drug is assembled to a specific patient. Can be an inpatient that is at the hospital receiving treatment, or an outpatient that comes to the Pharmacy to pick up a prescription. It only varies on the way of requesting. But one way or another, the drugs are assembled and labelled to the specific patient, assuring its safety and that is the right drug, on the right moment, to the right patient.

To accomplish this, a team of pharmacists, technicians and assistants, led by a supervisor, work so that the flow of the drug from the arriving of the prescription until its supply is done properly and in time.

Not only this is a space of work, but is also a space to store the DU's Qty from boxes that were split up during dispensing or came back from the returns as part packs.

Briefly, a prescription arrives, is checked by a pharmacist if needed, a label is printed, the drug is dispensed, double checked and delivered (3.1 and 3.2).

Dispensary is the middle point between a drug and the person meant to have it, playing a critical role of responsibility where the health of the patient is the priority and the pharmaceutical knowledge and care are the tools.

3.1 Prescription arrives

There are four main types of prescriptions that can arrive.

- The POD sheet
  - Arrives from the wards via *wooshie* or delivered by the pharmacist or technicians
  - Has written the specific drugs needed for the patients on that ward.
- Inpatient chart
  - Arrives from the wards via *wooshie* or delivered by the pharmacist or technicians
  - Usually requests all the drugs prescribed for that patient specifically
- TTO and Electronic Discharge System
  - Arrives from the wards via *wooshie* or delivered by the pharmacist or technicians
  - Has written the drugs that the patient will continue to have after leaving the hospital, and usually is supplied enough medication for a month

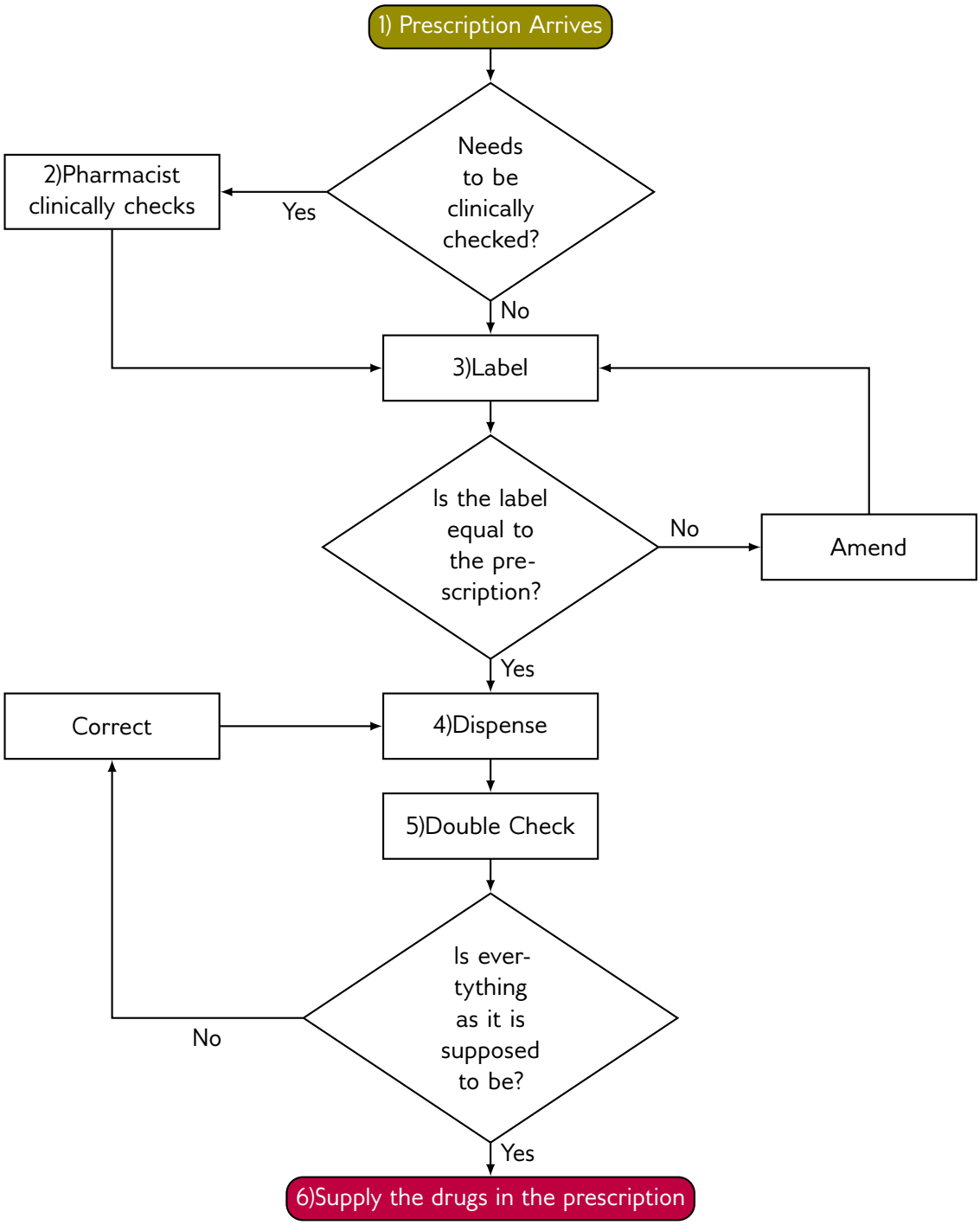


Figure 3.1: How to dispense. The numbers in the flowchart find a correlation with the numbers in the figure 3.2.

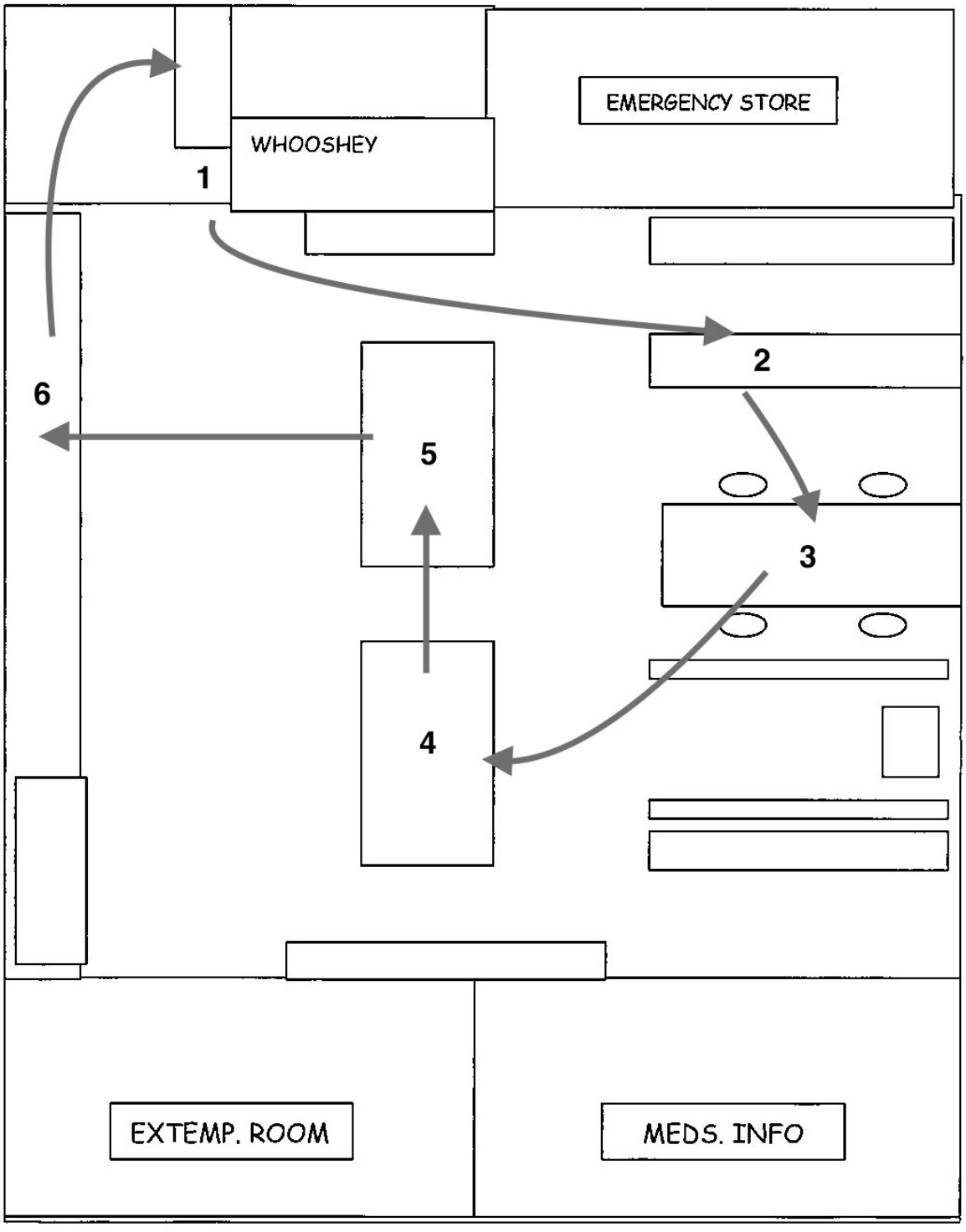


Figure 3.2: Dispensary blueprint and prescription movement. 1-Arrival of a prescription at the shop or by whooshey, 2-Clinical check, 3-Labelling, 4-Dispensing, 5-Double check, 6-Delivery.

- Outpatients prescription
  - Arrives at the shop from the outpatients that can wait for the drug or ask to call back
  - Has written the drugs that were prescribed from an outpatient appointment. Sometimes, can be a private patient and in those cases, they have to pay an extra for the dispensing work.

When there is more than one type of prescription to assembled, the outpatients are always the priority because they are waiting for the service to be completed.

3.1.1 Shop

The shop is the front face of the pharmacy where the outpatients can come and ask for a drug in a prescription and ask any question about medication. Staff from the hospital can come also to deliver prescriptions or ask for drugs that are urgent.

The outpatients might have to pay the prescription, contrary to an inpatient and that happens in the shop too. There is a list of situations when an outpatient is exempt to pay.

Sometimes, a patient can come to the shop to by OTC without a prescription and a clinical assess is made for the need of that drug and the patient situation.

3.2 Clinical Check

There are some prescriptions that are not clinically checked before arriving the dispensary - the outpatients and some TTO for the discharged patients.

The pharmacist is, therefore, responsible to look to the prescription and decide if it is clinically appropriate for the patient, gathering information if needed. The pharmacist can call the patient, call the consultant, check the GP records or search pharmaceutical information in specific bibliography in order to support their decision.

3.3 Labelling

Since the drug is specific for a patient, each label is written with personal information of the patient and the directions of the prescriber. A correct label 3.3 includes some transversal information to all the labels, like

- Patient's name
- Drug's name, including brand in some occasions
- Strength
- Pharmaceutical form
- Posology
- Quantity inside

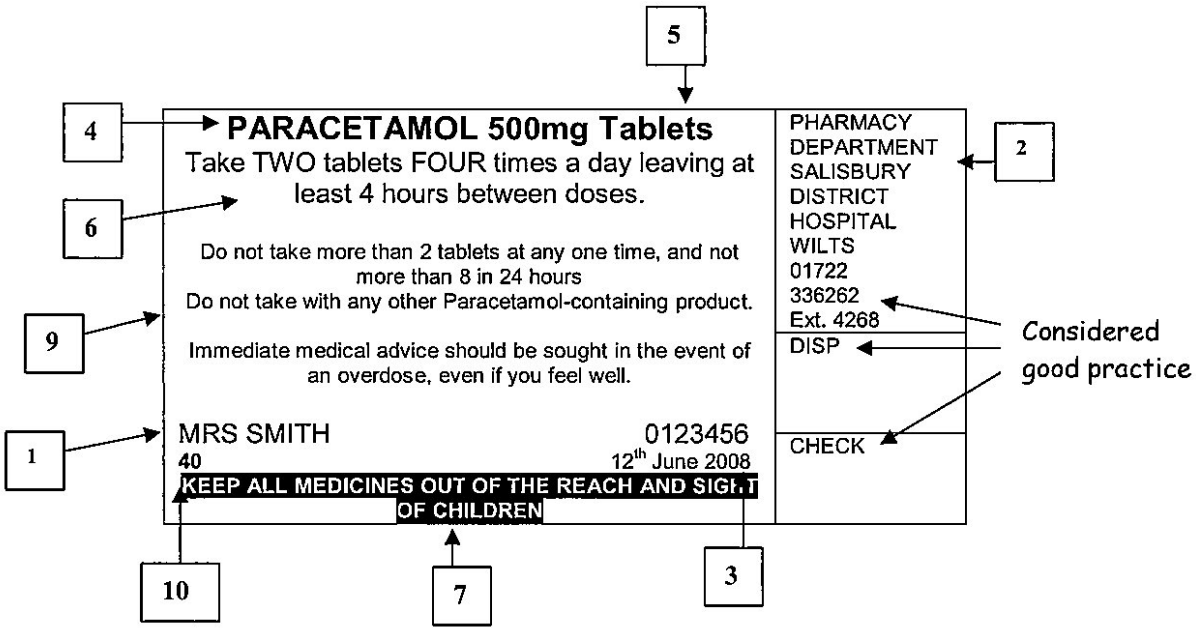


Figure 3.3: Label example. 1-Patient's name, 2-Hospital information, 3-Date of dispensing, 4-Name of the product, 5-Pharmaceutical form, 6-Direction of use, 7-"Keep all medicines out of the reach and sight of children" sentence, (8-Storage requirement), 9-Warnings, 10-Quantity dispensed.

- Hospital information
- Date of Dispensing
- "Keep all medicines out of the reach and sight of children" sentence

but can also include some extra information like possible allergies, care with the drug, warning for specific side effects, expiry date after opening, and much others, that are printed and stick along side with the main label.

Each label has also space for two signatures: one for the person who assemble the drug and another for the person who does the final check. This will be discussed later.

The labels are prepared via JAC, and the warnings needed for that medication pop out when creating that label specifically, allowing the user to select only the correct warnings, and to not forget anything. The indications are written with short keys, that then are expanded in a full text. When the person is happy with the label and believes that it matches what is in the prescription, prints the label and place it in a tray. When the drug needed is a full pack that is kept in the robot, the robot will take the drug automatically and the belt will carry the box to the tray. If not, the box needs to be picked up from its living place.

3.4 Dispensing

When the labels are ready, starts the dispensing. The medication is checked against the prescription and each label is stick to the respective box. When a full box is needed, the process is simple, but there are other situations that need more attention from the personnel dispensing.

3.4.1 Dispensing DU's Qty

When the patient does not need the quantity of a full box, the secondary packaging can be changed to accommodate the right amount. This includes cut blister from the original package or multiple boxes in just one big new package. Every time the original box is changed, the expiry date must be explicit. So, if it was cut and does not have the expiry date, an extra label must be added with the expiry date for the drug.

There are other situations when the packaging has to be changed. For example, when the tablets or capsules are inside a bottle that is not children proof, it is good policy to change them to a new bottle with a special lid to protect the children, except if the bottle has a desiccator to protect the drug. Usually this bottles are amber colour so it can protect the drug from light.

3.4.2 Dispensing liquids and temporary suspensions

A patient does not need always a full bottle of the medication. In those cases, the prescription has written the volume to dispense or the time course of that medication, and a person has to measure that volume and transfer it to a new bottle. The expiry date is six months from the opening of the bottle, with some exceptions.

Sometimes, it might be needed to reconstruct a temporary suspension. In this case, the person has to measure the correct amount of sterile water that is written in the packaging and add it to the powder that is going to be reconstituted. In some cases, the medication has the bottle with the powder and another one with the liquid, and in that case, is just add one to another and shake.

3.4.3 Dispensing fridge items

These items are dispensed like all the others, but must be kept in the fridge between the dispensing and the double check. A sheet saying that the item is stored in the fridge is placed in the tray so the double checker knows where is the medication. Usually, the label has already the warning to keep it in the fridge. If that is not the case, a small label has to be stuck to alert the patient.

3.4.4 Dispensing CD

The CD are kept in a locked cupboard and can only be dispensed by trained personnel with clearance for that. It has the particularity that every action must be written, and a record of the movement of the CD has to be kept.

3.4.5 Leaflets and signatures

When the drug is for the patient — TTO or outpatient — and not the ward, is compulsory to give the leaflet, even if it is not dispensed a full box. The patient has to have the information

about the drug he is taking, and in case there are no leaflet inside the box because it was used already for another part pack, it can be printed a new one from the leaflet database of the pharmacy.

At the end of the dispensing, the prescription must be signed near each item dispensed, as also the amount dispensed for each. The labels have to be all signed as well.

3.4.6 TF

When a medication is prescribed and there is no stock available in the pharmacy, it creates a TF and the pharmacist should be warned in case of a urge need of that drug for the patient.

Every TF is sorted individually. The most common is to wait for the drug to arrive on the next order. But if it is really needed, the strength can be changed fulfilling the dose prescribed – example: instead of a tablet of 100mg it is given two tablets of 50 mg –, or the formulation is changed – example: instead of capsules it is given tablets –.

A copy of the POD sheets is kept every time there is a TF.

When the drug arrives, is it labelled and given to the patient.

3.5 Double Check

After the dispensing, the tray with the labelled medication is kept on hold, queuing according with their priority, to be double check by someone trained for that, usually a pharmacist or technician. They check everything from the prescription, going throw the label and finally the medication and expiry date. If they agree it is correct, they give it to the patient, or call him saying that the prescription is now assembled and ready to pick up.

3.6 Giving the medication to an outpatient

When dispensing a prescription for an outpatient, the person has the opportunity to talk to him and ask and answer everything that might help the patient on how to rationally use the medication. The person can give all the indications, alerts for common side effects and give personalized information, proper for the patient in front of him.

Chapter 4

Other tasks performed

4.1 EDC

The EDC is a room outside the pharmacy where are stored some selected medicines in low quantity in order to be used by the staff when they are needed and the pharmacy is closed. Although there is a designated bank pharmacist for any purposed related with drugs out of pharmacy hours, the EDC avoids calling them for minor situations like dispensing a drug.

When a drug is taken, it should be written the drug taken, for who is it for – hospital number–, and the consultant. The medication there kept covers the majority of the drugs used at the hospital.

Someone responsible for the EDC books out the drugs taken during the night to the correct patient.

Every medication kept in the EDC must have a label saying "Emergency Cupboard", so the pharmacist knows where does the medications comes from and why does it not have a label with the patients information.

4.2 Leeches

Leeches are part of the biotherapy used in the hospital, and they are cared by the pharmacy. They are kept in the refrigerator, but once a week, they are cleaned and their water is changed.

The water for the leeches can not be common water for some ions are too aggressive for the, and can not be wither distilled water because the ions from the leeches would flow to the water leaving them without any. So, a brand salt called "Hirudo" has the right ions in the right proportions for the leeches.

They are kept in individual pots with a small cap to avoid them to run away.

There are leeches that are kept in the EDC and must be taken care too.

Every time a leech is used, it can not be reused for a different patient for safety reasons and must be sacrificed.

4.3 CD destruction

All the CD – usually drugs that create addiction – must be destroyed before being disposed. They are kept in a locked cupboard until they are enough to make the procedure. The tablets are crushed and the capsules are opened, and they are disposed to the "DenKit", a kit with a powder than in water, creates a solid and rigid mortar preventing the drugs to be taken from there.

4.4 Wards

A part of my placement consisted in shadowing MMT, pharmacist and nurses on the wards to learn how to deal with patients, their problems and medication issues.

I went to different wards with different professionals and I watched them on a variety of tasks like:

- Accessing GP records
- Checking the patients' PODs
- Questioning patients about how they take their medication
- Searching on the British National Formulary (BNF) and other data bases which drug would suit best the patient
- Interacting with doctors and other members of the team
- Reconciliation of the medication
- Create TTO for a patient discharged
- Discovering mistakes on the drug charts and correcting them

Even thought I could not spend a lot of time there, I appreciate a lot the experiences I had on the wards.

Chapter 5

Conclusion

These months at Salisbury NHS allowed me to see and fully understand the role of Stores and Dispensary.

I fell that I accomplished the goals planned by the Pharmacy for this placement, and I like to believe that I made a difference at some points and that my reflections and suggestions with the KROB and Returns will be helpful for the Pharmacy.

The placement did not corresponded to my expectations, since I was expecting to have a more deep training in pharmacy matters, but at least I was able to improve my English, deal with drugs that are strict to the hospital environment, and share experiences and opinions with other health professionals.

I would like to thank all of those who in a way or another tried to make my day better and taught me all they could. The knowledge I acquired during the placement is vast and I will try to implement it on my future, either to strength an existing role or to open doors to a new one.

Appendices

Appendix A

Creating the KROB spreadsheet

As said before, KROB spreadsheets are a fast and easy way to analyse stock levels from products that live inside the Robot. In this appendix A, I will explain the path to create a good spreadsheet with some screenshots. All the data is fake and was chosen so it would represent the majority of examples that can appear.

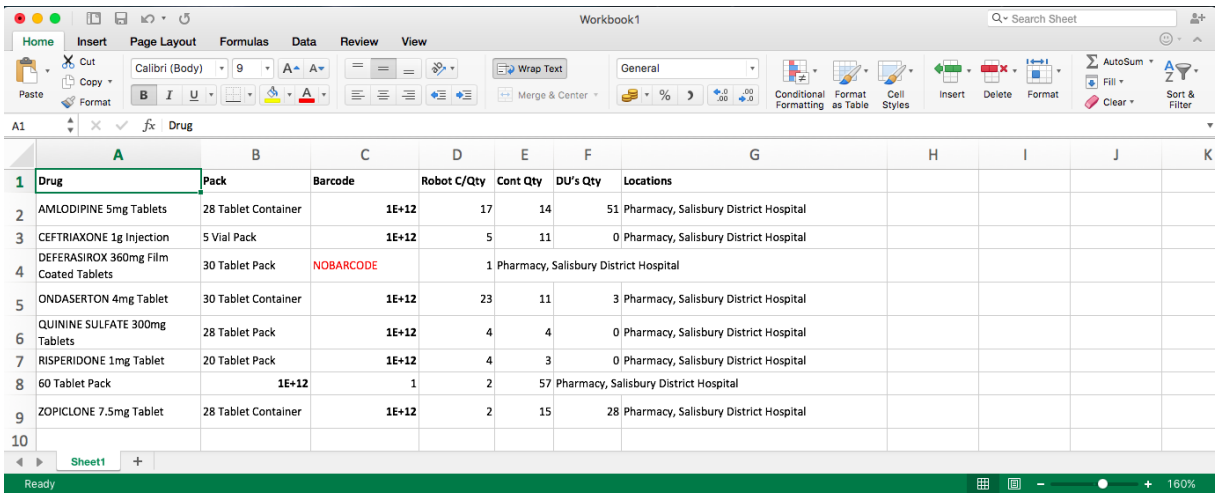
Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	Locations
AMLODIPINE 5mg Tablets	28 Tablet Container	000000000001	17	14	51	Pharmacy, Salisbury District Hospital
CEFTRIAXONE 1g Injection	5 Vial Pack	000000000002	5	11	0	Pharmacy, Salisbury District Hospital
DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE		1		Pharmacy, Salisbury District Hospital
ONDASERTON 4mg Tablet	30 Tablet Container	000000000003	23	11	3	Pharmacy, Salisbury District Hospital
QUININE SULFATE 300mg Tablets	28 Tablet Pack	000000000004	4	4	0	Pharmacy, Salisbury District Hospital
RISPERIDONE 1mg Tablet	20 Tablet Pack	000000000005	4	3	0	Pharmacy, Salisbury District Hospital
	60 Tablet Pack	000000000006	1	2	57	Pharmacy, Salisbury District Hospital
ZOPICLONE 7.5mg Tablet	28 Tablet Container	000000000007	2	15	28	Pharmacy, Salisbury District Hospital

Figure A.1: KROB table

As shown is A.1, the KROB list gives information about the items:

- Name of the drug
- Pack size
- Barcode
- Amount of items inside the ROBOT, said by it
- Amount of items JAC says that exist
- DU's Qty, which means the packs that are not full and usually are kept in dispensary
- Location

The first step is to copy the full table (A.1) from a Word document to an Excel document (A.2). It will happen that it will lose the formatting and some particular problems will appear. For example, when there is a blank space in the Word document, the Excel will ignore it and



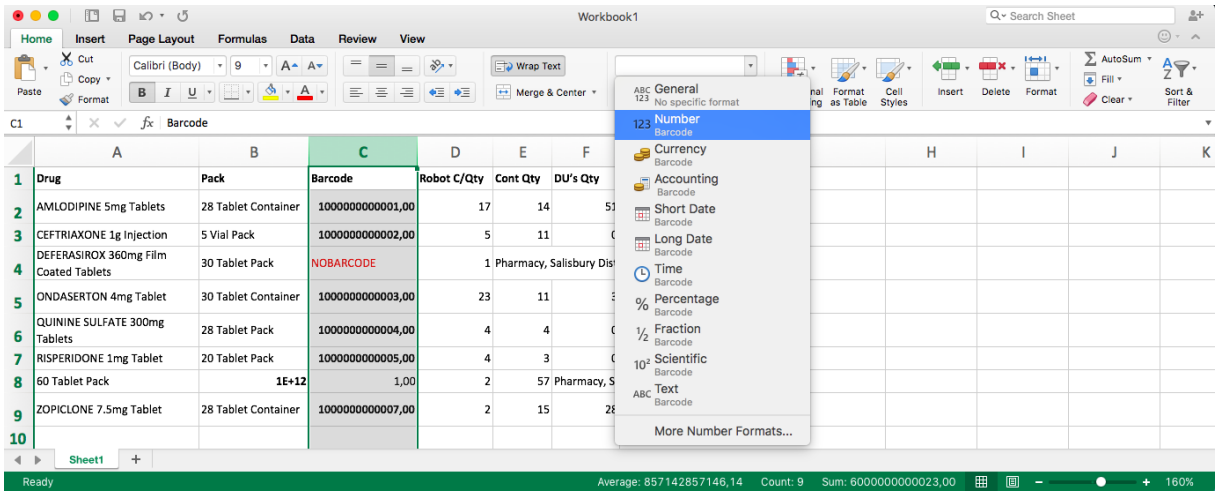
	A	B	C	D	E	F	G
1	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	Locations
2	AMLODIPINE 5mg Tablets	28 Tablet Container	1E+12	17	14	51	Pharmacy, Salisbury District Hospital
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1E+12	5	11	0	Pharmacy, Salisbury District Hospital
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE	1	Pharmacy, Salisbury District Hospital		
5	ONDASERTON 4mg Tablet	30 Tablet Container	1E+12	23	11	3	Pharmacy, Salisbury District Hospital
6	QUININE SULFATE 300mg Tablets	28 Tablet Pack	1E+12	4	4	0	Pharmacy, Salisbury District Hospital
7	RISPERIDONE 1mg Tablet	20 Tablet Pack	1E+12	4	3	0	Pharmacy, Salisbury District Hospital
8	60 Tablet Pack	1E+12	1	2	57	Pharmacy, Salisbury District Hospital	
9	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1E+12	2	15	28	Pharmacy, Salisbury District Hospital
10							

Figure A.2: Import the data to a spreadsheet

write the next column on the previous space, as shown in the Deferasirox row and Risperidone 60 Tablet Pack.

The next steps are in this order for personal taste, and do not need to be followed in this order.

I like to correct the Barcode column first to see the full barcode, changing the cells category to number (A.3). Some product don't have a barcode defined and it will appear in red letters, just like in Deferasirox. In that cases, it can be changed on JAC by someone with clearance for that.



	A	B	C	D	E	F
1	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty
2	AMLODIPINE 5mg Tablets	28 Tablet Container	1000000000001,00	17	14	51
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1000000000002,00	5	11	
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE	1	Pharmacy, Salisbury Dist	
5	ONDASERTON 4mg Tablet	30 Tablet Container	1000000000003,00	23	11	
6	QUININE SULFATE 300mg Tablets	28 Tablet Pack	1000000000004,00	4	4	
7	RISPERIDONE 1mg Tablet	20 Tablet Pack	1000000000005,00	4	3	
8	60 Tablet Pack	1E+12	1,00	2	57	Pharmacy, S
9	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1000000000007,00	2	15	28
10						

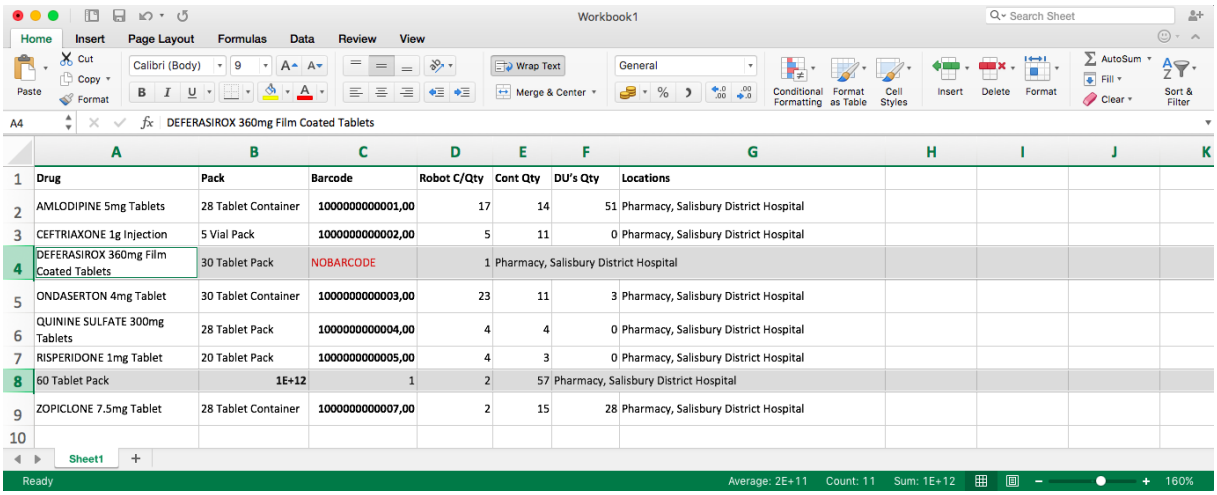
Figure A.3: Format Barcode column

After that, I correct the blank gaps errors (A.4) adjusting the information to the right columns. It can be a long process when there are many items in this situation, so it should be prioritise the ones that don't match between Robot quantity (Robot C/Qty) and JAC quantity columns.

In Deferasirox Row, the KROB list does not have the number zero for the Robot C/Qty, and the Excel skipped it. It is important to check with the original list because we can not know for sure in which row that number was previously and that is a big different if we are

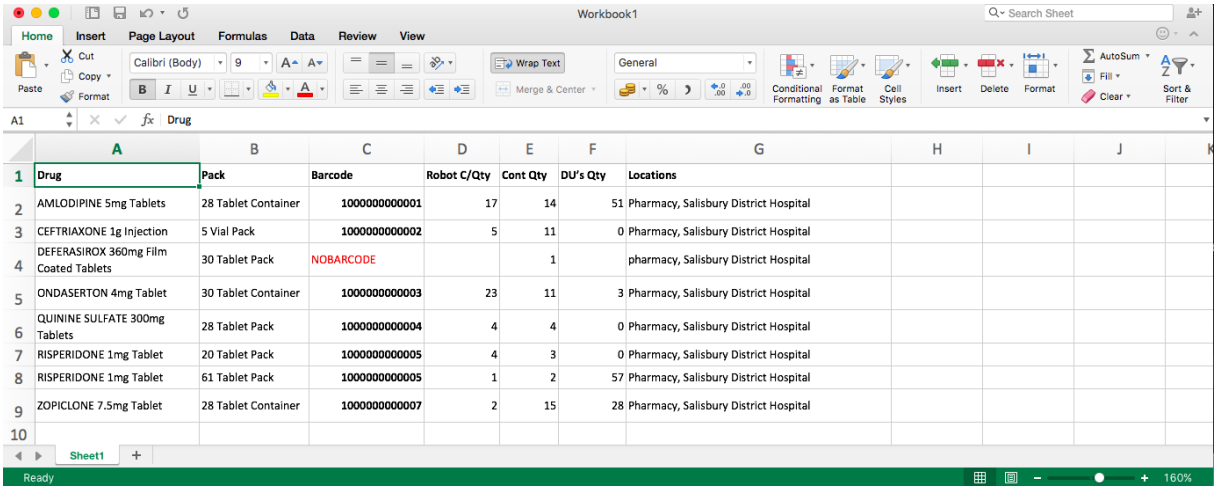
short in one box or if we have one more.

In the Risperidone row, the Excel did not understand that the pack size refers to the previous item. The best to do is to change all the information one cell to the right manually and copy the name from the drug. The final result is in figure A.5.



	A	B	C	D	E	F	G	H	I	J	K
1	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	Locations				
2	AMLODIPINE 5mg Tablets	28 Tablet Container	1000000000001,00	17	14	51	Pharmacy, Salisbury District Hospital				
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1000000000002,00	5	11	0	Pharmacy, Salisbury District Hospital				
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE	1	Pharmacy, Salisbury District Hospital						
5	ONDASERTON 4mg Tablet	30 Tablet Container	1000000000003,00	23	11	3	Pharmacy, Salisbury District Hospital				
6	QUININE SULFATE 300mg Tablets	28 Tablet Pack	1000000000004,00	4	4	0	Pharmacy, Salisbury District Hospital				
7	RISPERIDONE 1mg Tablet	20 Tablet Pack	1000000000005,00	4	3	0	Pharmacy, Salisbury District Hospital				
8	60 Tablet Pack	1E+12	1	2	57	Pharmacy, Salisbury District Hospital					
9	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1000000000007,00	2	15	28	Pharmacy, Salisbury District Hospital				
10											

Figure A.4: Gaps without the correct formatting



	A	B	C	D	E	F	G	H	I	J	K
1	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	Locations				
2	AMLODIPINE 5mg Tablets	28 Tablet Container	1000000000001	17	14	51	Pharmacy, Salisbury District Hospital				
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1000000000002	5	11	0	Pharmacy, Salisbury District Hospital				
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE		1		pharmacy, Salisbury District Hospital				
5	ONDASERTON 4mg Tablet	30 Tablet Container	1000000000003	23	11	3	Pharmacy, Salisbury District Hospital				
6	QUININE SULFATE 300mg Tablets	28 Tablet Pack	1000000000004	4	4	0	Pharmacy, Salisbury District Hospital				
7	RISPERIDONE 1mg Tablet	20 Tablet Pack	1000000000005	4	3	0	Pharmacy, Salisbury District Hospital				
8	RISPERIDONE 1mg Tablet	61 Tablet Pack	1000000000005	1	2	57	Pharmacy, Salisbury District Hospital				
9	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1000000000007	2	15	28	Pharmacy, Salisbury District Hospital				
10											

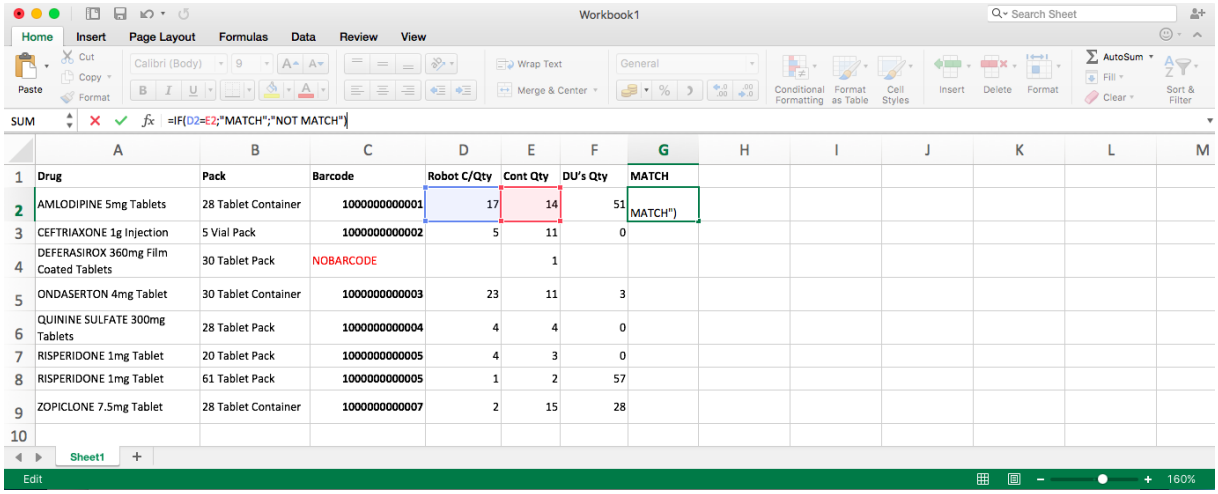
Figure A.5: Correct formatting in all rows

When this is all fixed, we can proceed to the analysis itself. I usually delete the column of location because it's not important for this stock check, and on column G I write the formula: =IF(D2=E2;"MATCH";"NOT MATCH"). This formula means that "if" the cell "D2" is "equal" to "E2" it writes "MATCH". If not, it writes "NOT MATCH" (A.6).

If we drag the corner of the cell, it will apply to every cell. As it can be seen in Figure A.7, the row of Quinine has 4 items in both columns Robot C/Qty and JAC quantity (JAC C/Qty), making a match — not a discrepancy.

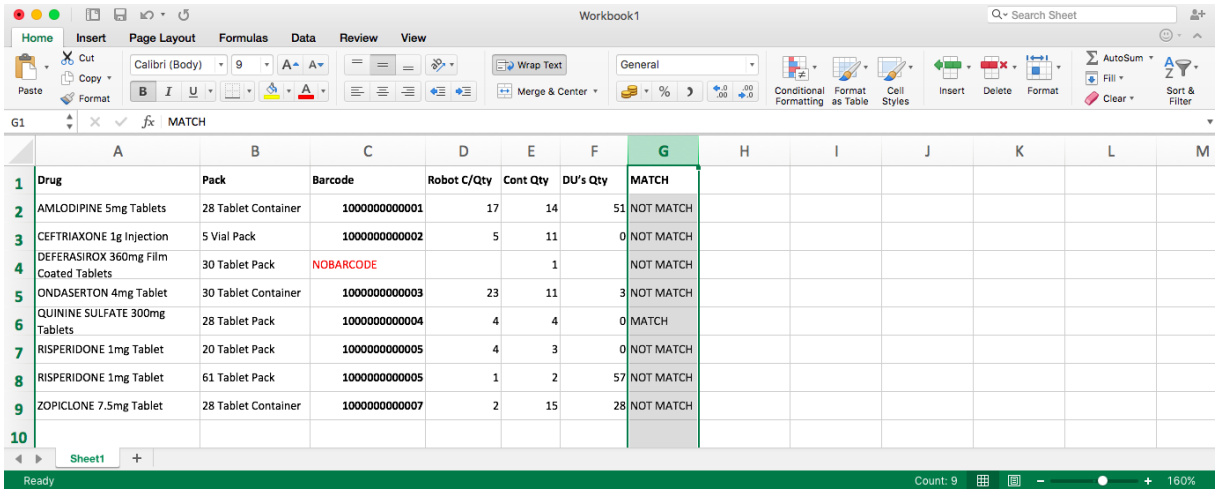
After that, it is important to know the value of the difference between every item, in order to see if the discrepancies are minor or major[1]. For that, on column H, I write the formula:

[1] I like to consider a major discrepancy when the difference is bigger then $+7$ or less then -7, but that is a matter of personal sensibility.



	A	B	C	D	E	F	G
1	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	MATCH
2	AMLODIPINE 5mg Tablets	28 Tablet Container	1000000000001	17	14	51	MATCH")
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1000000000002	5	11	0	
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE		1		
5	ONDASERTON 4mg Tablet	30 Tablet Container	1000000000003	23	11	3	
6	QUININE SULFATE 300mg Tablets	28 Tablet Pack	1000000000004	4	4	0	
7	RISPERIDONE 1mg Tablet	20 Tablet Pack	1000000000005	4	3	0	
8	RISPERIDONE 1mg Tablet	61 Tablet Pack	1000000000005	1	2	57	
9	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1000000000007	2	15	28	
10							

Figure A.6: Matching information and "IF" formula



	A	B	C	D	E	F	G
1	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	MATCH
2	AMLODIPINE 5mg Tablets	28 Tablet Container	1000000000001	17	14	51	NOT MATCH
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1000000000002	5	11	0	NOT MATCH
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE		1		NOT MATCH
5	ONDASERTON 4mg Tablet	30 Tablet Container	1000000000003	23	11	3	NOT MATCH
6	QUININE SULFATE 300mg Tablets	28 Tablet Pack	1000000000004	4	4	0	MATCH
7	RISPERIDONE 1mg Tablet	20 Tablet Pack	1000000000005	4	3	0	NOT MATCH
8	RISPERIDONE 1mg Tablet	61 Tablet Pack	1000000000005	1	2	57	NOT MATCH
9	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1000000000007	2	15	28	NOT MATCH
10							

Figure A.7: Matching information for all the cells

=(D2-E2), and drag the right corner of the cell down to add the formula to the other rows (A.8).

I also add the "Filter" option with the first row selected, because it will be needed in the next steps (A.9).

If the result in column H is positive, there are more items inside the robot than we have registered in the system. If the result is negative, there are more items in the system then inside the robot. With this, now we can create two big groups, and they must be investigated in different ways (A.10).

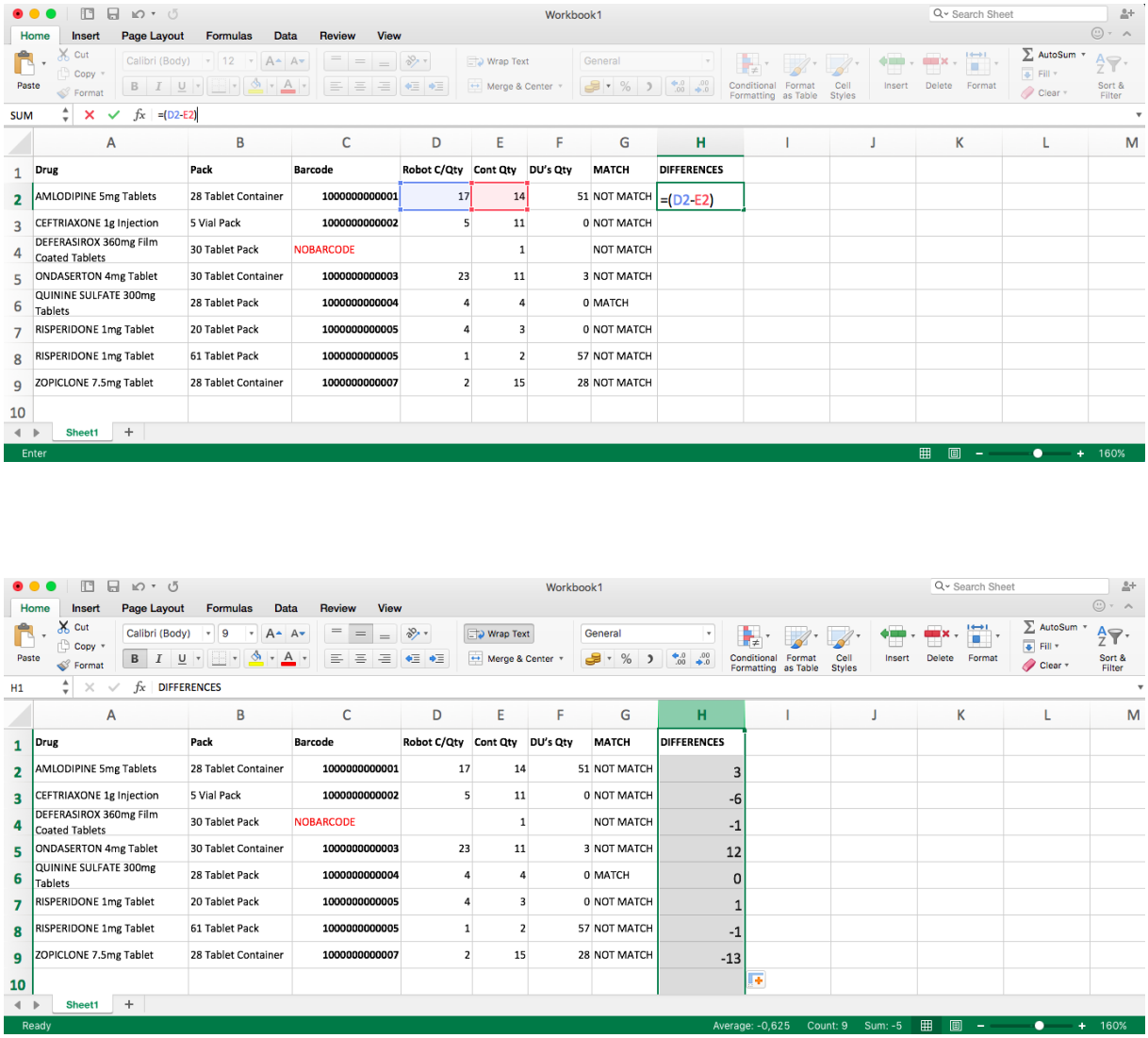


Figure A.8: Difference formula and expansion for all the rows

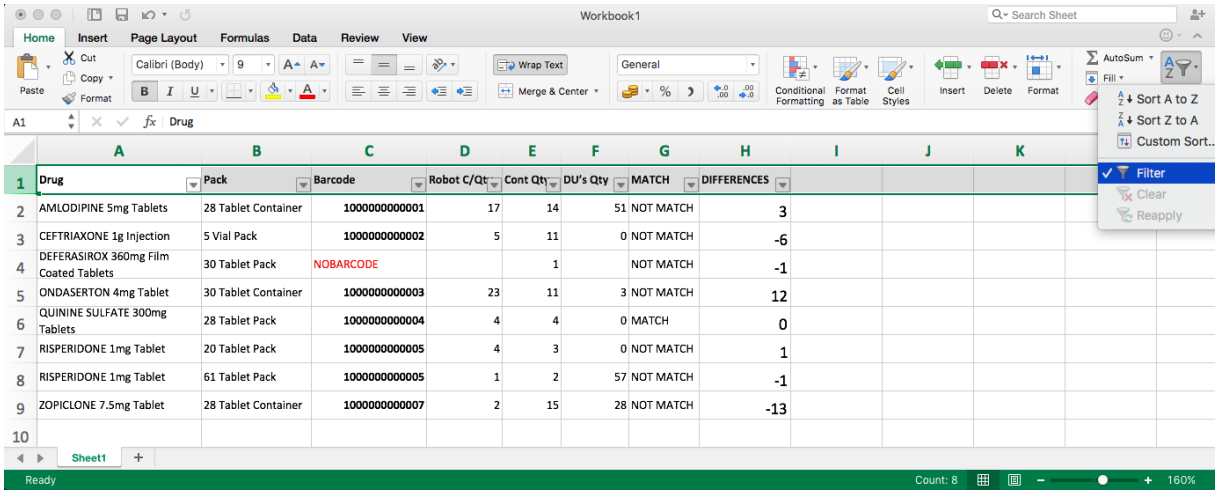
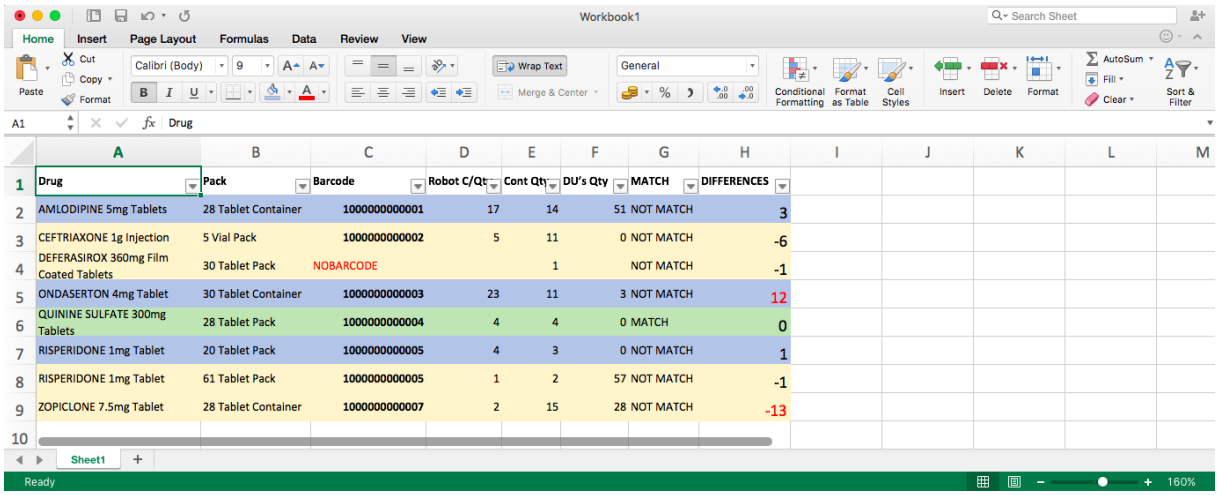


Figure A.9: Add a filter option

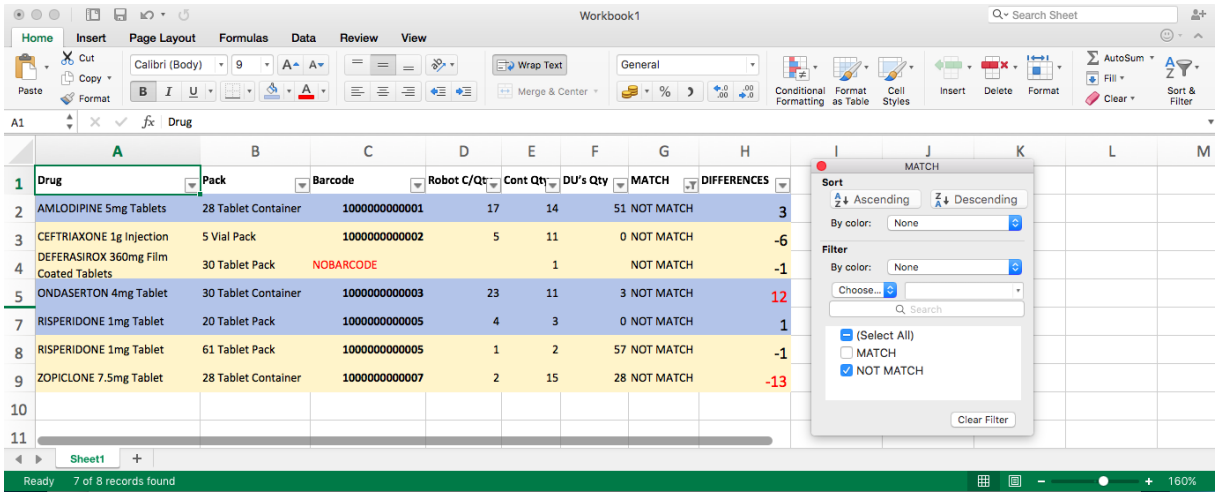
As we can see on figure A.10 [2], Quinine is a Match, Amlodipine, Ondarserton and Riperidone 20 Tablet Pack have a positive difference; and Ceftriaxone, Deferasinox, Risperidone 61 Tablet Pack and Zopiclone have a negative difference.



	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	MATCH	DIFFERENCES
2	AMLODIPINE 5mg Tablets	28 Tablet Container	1000000000001	17	14	51	NOT MATCH	3
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1000000000002	5	11	0	NOT MATCH	-6
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE		1		NOT MATCH	-1
5	ONDASERTON 4mg Tablet	30 Tablet Container	1000000000003	23	11	3	NOT MATCH	12
6	QUININE SULFATE 300mg Tablets	28 Tablet Pack	1000000000004	4	4	0	MATCH	0
7	RISPERIDONE 1mg Tablet	20 Tablet Pack	1000000000005	4	3	0	NOT MATCH	1
8	RISPERIDONE 1mg Tablet	61 Tablet Pack	1000000000005	1	2	57	NOT MATCH	-1
9	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1000000000007	2	15	28	NOT MATCH	-13

Figure A.10: Complete spreadsheet

We can now star to filter. I remove the Matches because they are not a problem (A.11).



	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	MATCH	DIFFERENCES
2	AMLODIPINE 5mg Tablets	28 Tablet Container	1000000000001	17	14	51	NOT MATCH	3
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1000000000002	5	11	0	NOT MATCH	-6
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE		1		NOT MATCH	-1
5	ONDASERTON 4mg Tablet	30 Tablet Container	1000000000003	23	11	3	NOT MATCH	12
7	RISPERIDONE 1mg Tablet	20 Tablet Pack	1000000000005	4	3	0	NOT MATCH	1
8	RISPERIDONE 1mg Tablet	61 Tablet Pack	1000000000005	1	2	57	NOT MATCH	-1
9	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1000000000007	2	15	28	NOT MATCH	-13

Figure A.11: Using filters to remove the matches

Then, I like to separate the negatives in one sheet, and the positive in another sheet. I apply the filter on the "Differences" column: "Greater Than" - "0" (A.12), and "Less then" - "0" (A.13).

[2]The colours were added manually to facilitate the interpretation in this example.

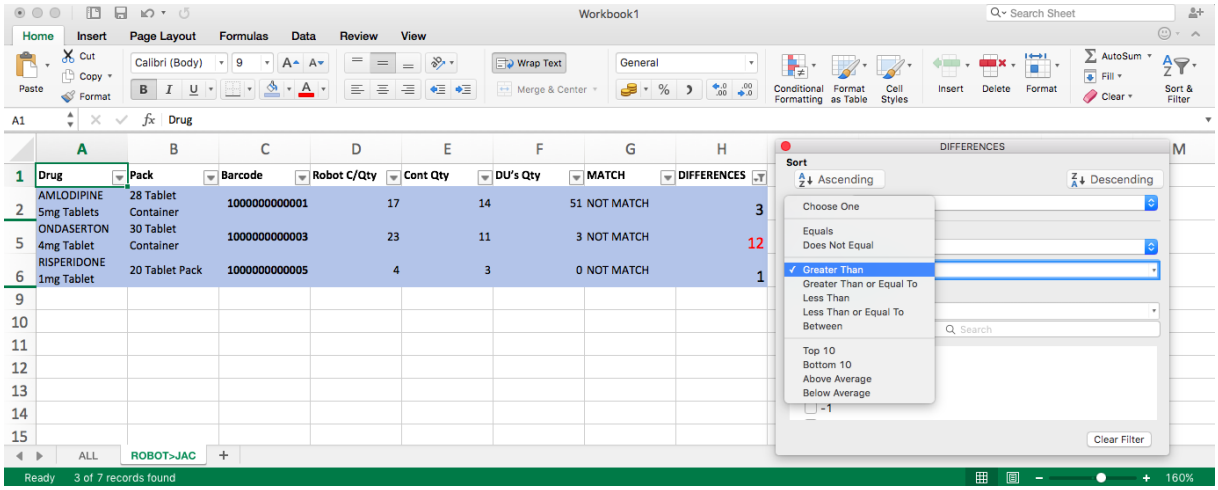


Figure A.12: Using filters to just see the positive differences

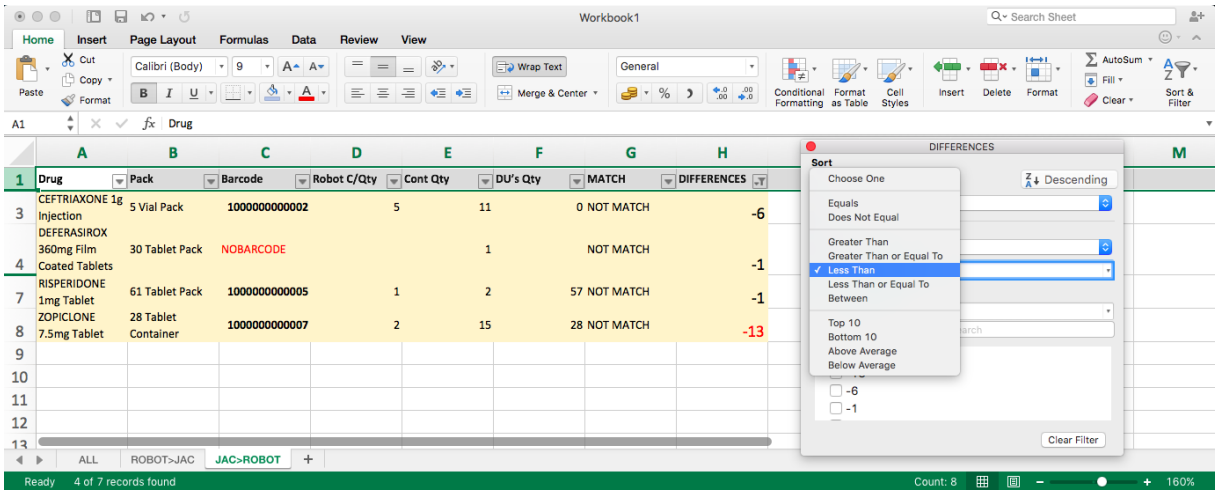


Figure A.13: Using filters to just see the negative differences

With this understood, there is a new detail I can add – the DU's Qty. DU's Qty represent a big part of stock and money that sometimes is ignore. But they can be the answer to some discrepancies, because we never know if some container was kept on the dispensary and opened. I like to create a forth sheet and copy all the "Not Match" items, and remove on the filter of the DU's Qty column the "zeros" and "blank spaces" (A.14).

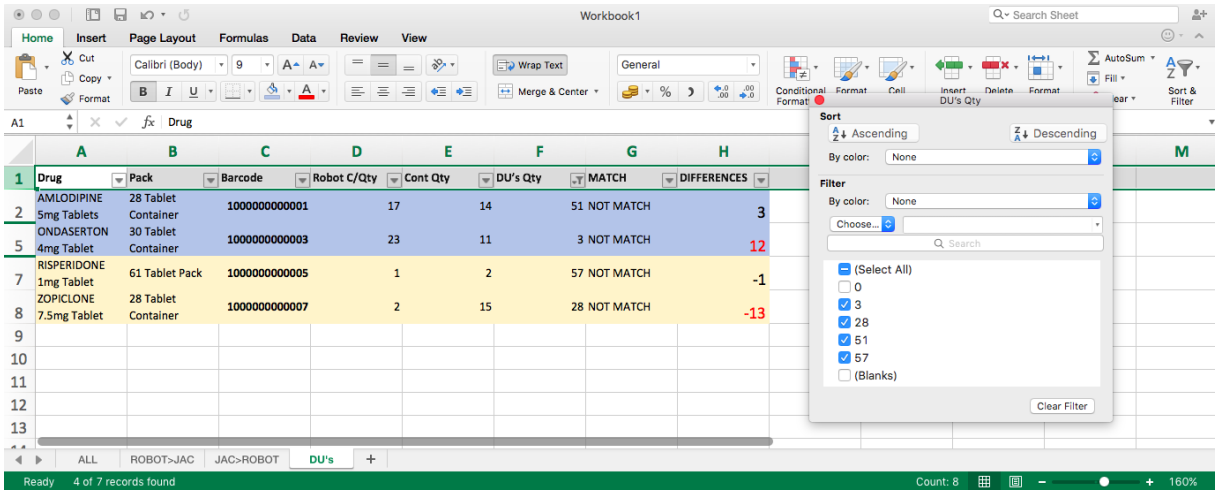


Figure A.14: DU's Qty filter

Now, I want to see how many full containers my DU's Qty represent. In the Pack size column I have that information, but we can not apply a formula with the text. To solve that, I copy the data to a new column and I go to "Data" – "Text to Columns". I select the "Delimiters" option and then I tick "Space" (A.15). Then, we can see it separated in three columns, but we just want the first, so we can click on the second and third column and select "Do not import column", and then hit the button "Finish" (A.16).

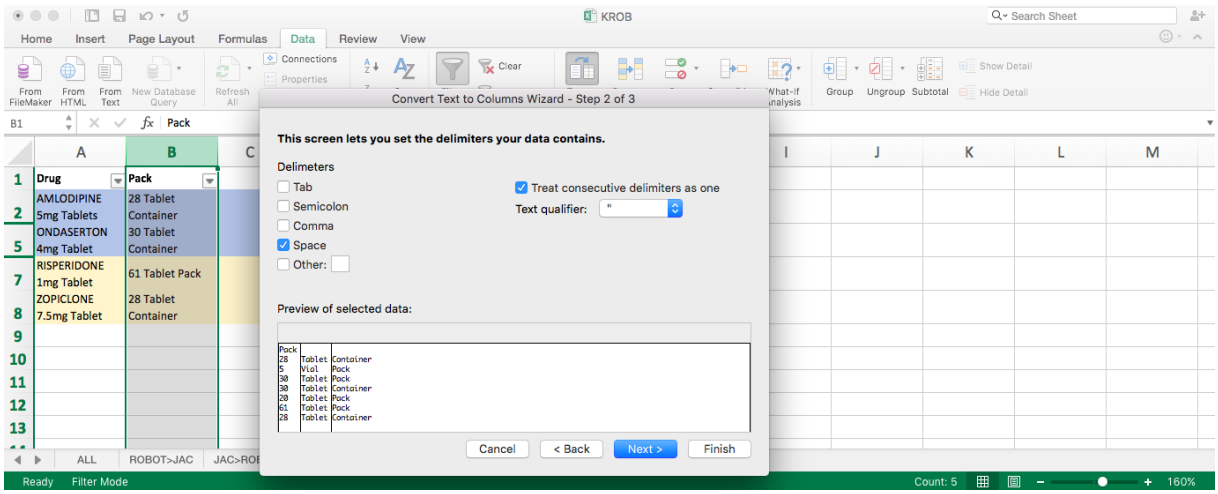


Figure A.15: Path to remove the text from the Pack size column.

With the numbers separated from the text, we can apply the formula =(G2/C2), that devises the DU's Qty with the pack size (A.17). Finally, we can round the numbers to remove the decimal digits by hitting the "Decimal Decrease" button (A.18).

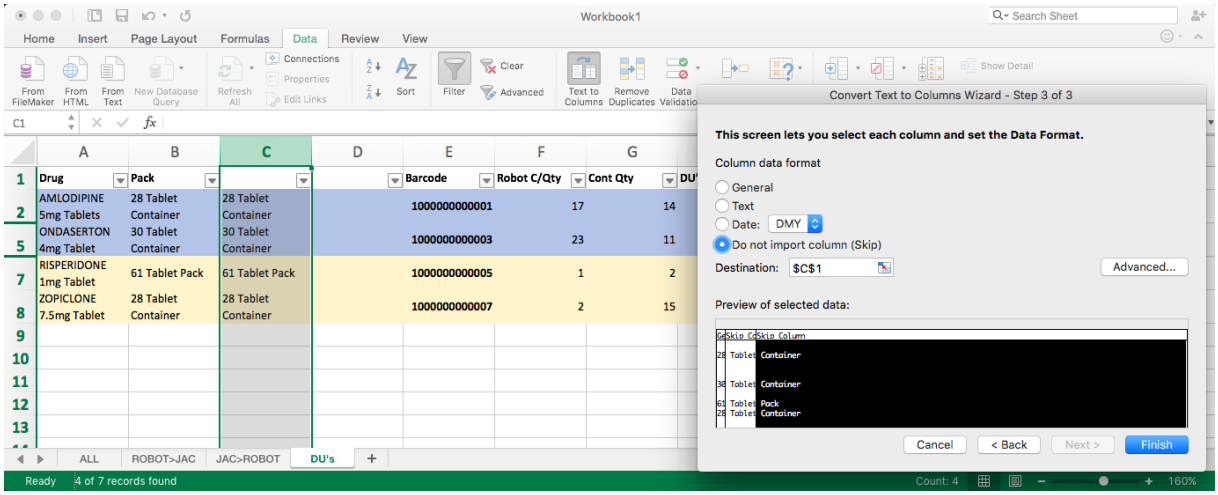


Figure A.16: Final options to optimize the data.

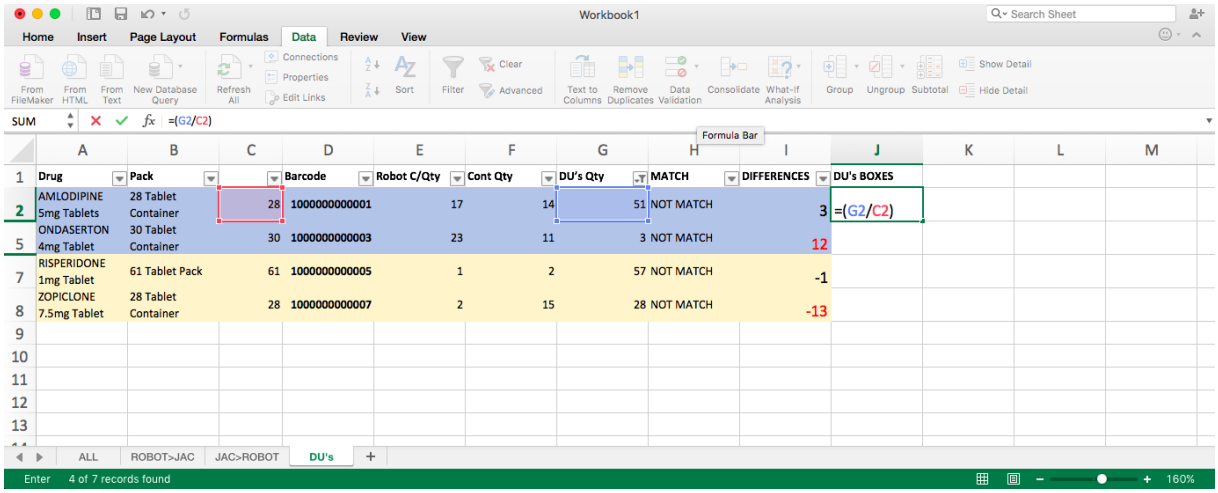


Figure A.17: Divide formula

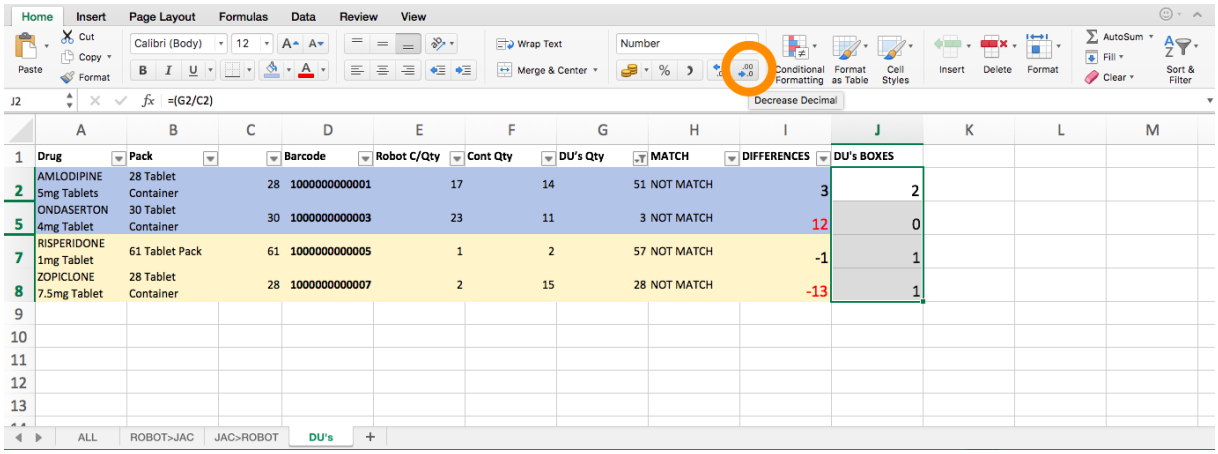


Figure A.18: Round numbers

We can now calculate the real difference between the JAC C/Qty and the real number of containers with the formula =(I2-J2) (A.19). This can resolve the discrepancy, lighten it or make it worse (A.20).

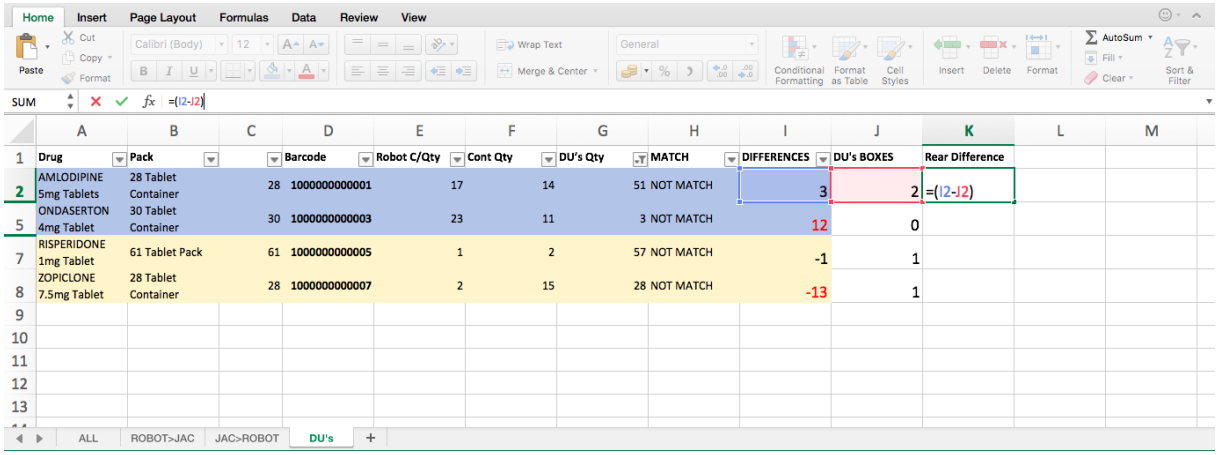


Figure A.19: Real difference formula

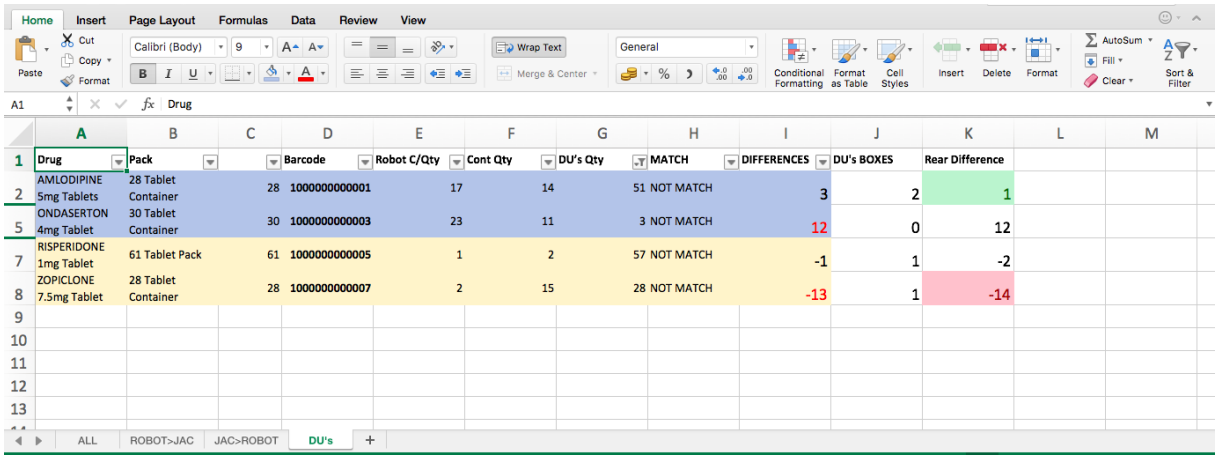
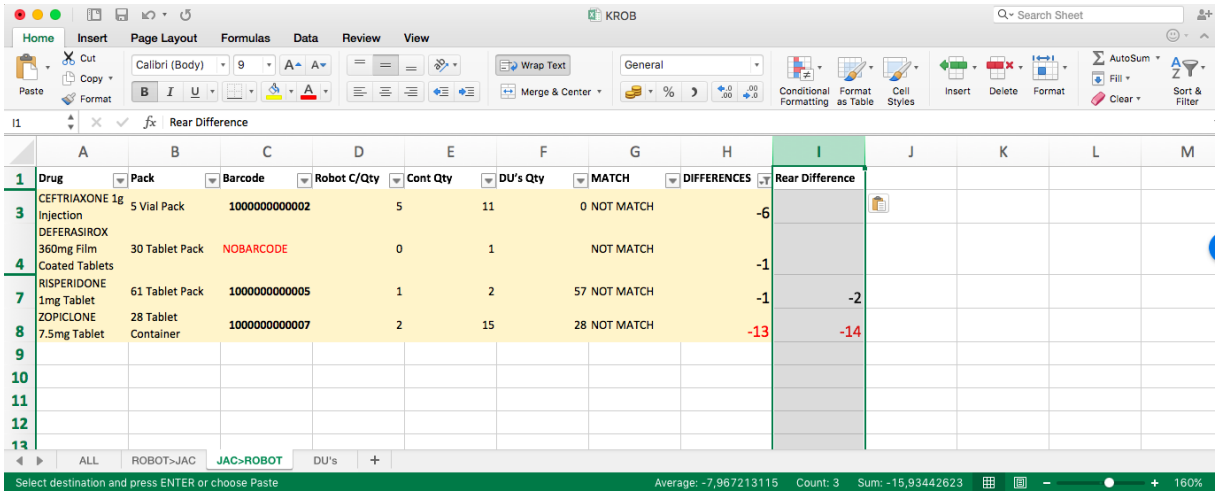


Figure A.20: Real difference

Several things can be made to improve the data formatting, like update the DU's Qty data result to the respect sheet or filter the rows with DU's Qty different from zero on the sheets "ROBOT>JAC" and "JAC>ROBOT". It depends on how the results will be seen and what we are looking for. I like to add the real differences to the sheets like in figure A.21.



	Drug	Pack	Barcode	Robot C/Qty	Cont Qty	DU's Qty	MATCH	DIFFERENCES	Rear Difference
3	CEFTRIAXONE 1g Injection	5 Vial Pack	1000000000002	5	11	0	NOT MATCH	-6	
4	DEFERASIROX 360mg Film Coated Tablets	30 Tablet Pack	NOBARCODE	0	1		NOT MATCH	-1	
7	RISPERIDONE 1mg Tablet	61 Tablet Pack	1000000000005	1	2	57	NOT MATCH	-1	-2
8	ZOPICLONE 7.5mg Tablet	28 Tablet Container	1000000000007	2	15	28	NOT MATCH	-13	-14

Figure A.21: Improving the sheets

The spreadsheet is now ready to be used to investigate the discrepancies one by one. Here are some final tips on how to interpret the results. This list is not exclusive, but might represent the majority of the cases:

- The Robot as zero quantity
  - Probably the item is not currently stored in the robot because of some change in the pack format that forced it to live in a new shelf, but it was not still updated on JAC;
  - The item was recently booked in but was not placed inside the robot yet
- There is a major discrepancy with Robot C/Qty bigger then JAC C/Qty [3]
  - The item was placed inside the robot but it was not booked in yet
- There is a major discrepancy with JAC C/Qty bigger then Robot C/Qty
  - The product was spat from the Robot for being out of date but it was not booked out yet
  - The item was wrongly booked in during the returns or receipt because of errors that happen with the pack size and the pack number
  - Not all the packs are living in the robot and have different locations
- Minor discrepancies
  - Some one placed/took an item manually inside/from the robot.

[3] They represent stopped money, because we have the item but we can not use it