

# **Vitamin D Point-of-Care Testing**

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of the requirements for the award of  
Doctorate in Pharmacy

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I dedicate my work to my family and friends, the source of sunshine in my life.

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## Abstract

Growing recognition of the multifaceted role of Vitamin D has augmented the demand for Vitamin D testing through swift, yet reliable, point-of-care testing (POCT) methods, unveiling a niche for development of a novel pharmacist-led service.

The aim was to establish a framework for pharmacist-led Vitamin D POCT in primary care. The objectives were to: 1) Review available Vitamin D POCT, 2) Validate the Vitamin D POCT versus the gold standard, 3) Develop and validate a framework for Vitamin D POCT and 4) Assess the feasibility of the pharmacist-led framework within community pharmacy.

The method consisted of: 1) Appraisal of Vitamin D POCT, 2) Validation of a Vitamin D POCT kit by comparing laboratory test results from Mater Dei Hospital (gold standard) with POCT results (20 patients), 3) Development and validation of a framework for pharmacist-led Vitamin D POCT, consisting of a Data Collection Sheet, Standard Operating Procedure and Action Plan and 4) Assessment of the feasibility of the developed framework (80 participants) within a community pharmacy setting.

1) Seven Vitamin D POCT kits were compared, 3 of which are available locally. The test kits use chromatographic immunoassay techniques providing quantitative (n=4) or semi-quantitative (n=3) results. The test selected for use in this study was the semi-quantitative AcroBiotech Inc. Vitamin D Rapid Test Cassette with a sensitivity of 4ng/ml and a cost of €6 per kit. 2) Concordance between the two methodologies was observed when the POCT kit was validated against the MDH lab value ( $\kappa = 0.84$ , p-value <0.001). 3) The Data Collection Sheet involves assessment of risk factors associated with development of Vitamin D deficiency. The Action Plan provides recommendation on maintaining adequate Vitamin D levels to patients through an Information Leaflet in English and

Maltese and guidance on supplementation to prescribers, as necessary. A Referral Note is used to refer patients to prescribers when Vitamin D deficiency is identified, for symptomatic patients or patients at high risk of developing Vitamin D deficiency. 4) Feasibility testing of the Vitamin D POCT framework within a community pharmacy setting was carried out on 80 participants, 8 participants having deficient and 49 participants insufficient Vitamin D levels. Significant association was observed between participant perception that Vitamin D levels have an important impact on general health with sun exposure ( $p = 0.034$ ). Participants who suffer from chronic conditions are more likely to have their Vitamin D levels tested ( $p = 0.042$ ), those suffering from metabolic disorders being more likely to have deficient/insufficient levels of Vitamin D ( $p = 0.026$ ). Participants who had their Vitamin D levels tested are more likely to be prescribed Vitamin D supplementation ( $p < 0.001$ ), to which they are adherent ( $p < 0.001$ ). Concurrent consumption of Vitamin D when taking other medication/supplementation was observed ( $p < 0.001$ ).

The review identified a POCT kit that could be used within a framework for community pharmacist-led assessment of Vitamin D within the context of collaborative care. Despite the study being carried out within a Mediterranean climate, a high incidence of Vitamin D deficiency/insufficiency was observed, indicating the value of providing access to this service. A pharmacist-led service would support patients who require referral, access to testing and early detection of Vitamin D deficiency/insufficiency.

Keywords: community pharmacy; pharmacist-led service; point-of-care testing; Vitamin D

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## **List of Abbreviations**

25(OH)D – 25-hydroxyvitamin D

BMD – Bone Mineral Density

BMI – Body Mass Index

CLIA – Chemiluminescence Immunoassay

CPBA – Competitive Protein-Binding Assay

GORD – Gastro-oesophageal Reflux Disease

GIOP – Glucocorticoid-Induced Osteoporosis

HPLC-MS – High-Performance Liquid Chromatography – Mass Spectrometry

HPLC-UV - High-Performance Liquid Chromatography – Ultraviolet Radiation

HRQoL – Health-Related Quality of Life

IVD – In-Vitro Diagnostic

IVDR – In-Vitro Diagnostics Regulation

LC-MS/MS – Liquid Chromatography tandem Mass Spectrometry

MDH – Mater Dei Hospital

NHS – National Health System

NICE – National Institute for Health and Care Excellence

POCT – Point-of-Care Test

RCT – Randomised Control Trial/s

RIA – Radioimmunoassay

RMP – Reference Measurement Procedure

SOP – Standard Operating Procedure

UV – Ultraviolet Radiation

UV-B – Ultraviolet Radiation Type-B

VDSP – Vitamin D Standardisation Program

## **Chapter 1 – Introduction**

## **1.1 Accessibility and Feasibility of Vitamin D Testing**

The implication that Vitamin D levels have a central role in a multitude of disease states is widely accepted (Felcher et al., 2017, Shah et al., 2018; Ebeling et al., 2018, Tsuprykov et al., 2018; Amrein et al., 2020; Bonnici et al., 2020; Gordon et al., 2020). Adequate levels of Vitamin D are considered an “excellent marker of good health” (Ebeling et al., 2018). This notion is complemented by a drastic rise in Vitamin D screening and testing, accompanied by prescriptions for Vitamin D supplementation for low Vitamin D across the globe (Felcher et al., 2017; Patel et al., 2020). Epidemiological studies carried out in the past decade have indicated that insufficient levels of Vitamin D are prevalent across the general population (Garg et al., 2019).

Vitamin D is a fat-soluble vitamin with key involvement within calcium homeostasis and bone metabolism, alongside other metabolic states, and cellular activity outside the skeletal system. A series of controversies concerning its clinical implications are persistent (Ebeling et al., 2018; Gorey et al., 2019; Krist et al, 2021). Global consensus regarding the exact serum levels of Vitamin D associated with sufficiency has not been met (Bonnici et al., 2020; Krist et al., 2021). In accordance with data published by the National Academy of Medicine (USA) in 2021, “97.5% of the population will have their Vitamin D levels met at a serum level of 20 ng/mL (49.9 nmol/L) and risk for deficiency, relative to bone health, begins to occur at levels less than 12 to 20 ng/mL (29.9- 49.9 nmol/L)” (Krist et al, 2021).

The Vitamin D thresholds proposed for serum 25(OH)D levels by the National Osteoporosis Society (UK) are in line with the Institute of Medicine (USA) which suggest

that Vitamin D levels less than 30 nmol/l (8.6 ng/mL) are deficient, values of 30–50 nmol/l (8.6 ng/mL – 14.4 ng/mL) may be inadequate in patients at risk for developing Vitamin D deficiency and measurements above 50 nmol/l (14.4 ng/mL) are sufficient for almost the whole population (Aspray et al., 2014; Francis et al., 2015). In Malta, serum 25(OH)D reference ranges follow those proposed by the Endocrine Society (USA) guidelines, where levels above 30ng/ml are considered adequate, levels between 20-30ng/ml are regarded as insufficient and levels below 20ng/ml are deemed deficient (Holick et al., 2012; Bonnici et al., 2020).

There has been much debate about which form of Vitamin D is to be measured and which levels constitute to Vitamin D deficiency, and different institutions have published varied limits with regards to the interpretation of Vitamin D assays (Amrein et al., 2020). A consensus that “serum 25(OH)D is considered to be the best marker for assessing Vitamin D status”, stands on a global level. This biomarker “reliably reflects the free fractions of the Vitamin D metabolites, despite the fact that, in theory, the bioavailable fractions may be more clinically informative” (Amrein et al., 2020).

The availability of a rapid, yet reliable method of assessing Vitamin D levels has become important in healthcare (Shah et al., 2018). Point-of-care testing (POCT) offers an alternative to traditional methods of laboratory evaluation, allowing the assessment of biomarkers at the patient’s bedside facilitating the clinical decision-making process at all strata of healthcare (Hohmeier et al., 2018). The complexity of the manner with which Vitamin D metabolites bind to Vitamin D binding protein (DBP) has rendered the analysis of Vitamin D a multifaceted feat since very small amounts of Vitamin D are found in the

unbound, “potentially biologically active” form (Tsuprykov et al., 2018; Bouillon et al., 2020).

Over the years, multiple methods for qualitative and quantitative analysis of Vitamin D have been developed and refined. Various techniques including, but not limited to, immunoassays and chromatography-based assays have been explored (Shah et al., 2018). These methods of analysis have been adopted and modified by biopharmaceutical companies in the production of Vitamin D POCT kits which allow for minimally invasive diagnostic capability, offering quantification of Vitamin D levels within minutes. The coupling of these POCT with non-invasive screening tools for Vitamin D deficiency, which take into consideration risk factors for developing Vitamin D insufficiency, obviates “unnecessary supplementation and blood testing” (Deschasaux et al., 2016). In turn, such actions contribute to access to Vitamin D testing and a reduction in workload on central medical laboratories (Gordon et al., 2020). Against this background, frameworks to establish feasibility and robustness of running point-of-care Vitamin D testing in community pharmacy needs investigation.

## **1.2 Methods of Analysis of Vitamin D**

“Serum total 25-hydroxyVitamin D (25(OH)D) is a measure of the total circulating 25(OH)D concentration, defined as the sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>. Serum 25(OH)D concentration is the primary measurement for evaluating Vitamin D status” as it is the major circulating form of Vitamin D, reflecting both dietary and cutaneous contributions having a half-life of 2-3 weeks (Bjerg et al., 2019). Measurement of active metabolite of Vitamin D, 1,25-dihydroxyvitamin D may give a false clinical picture in



patients with altered metabolic states such as secondary hyperparathyroidism. Alternative assays which complement measurement of serum 25(OH)D include serum 1,25(OH)<sub>2</sub>D, parathyroid hormone or markers of bone turnover as well as corrected serum calcium when severe Vitamin D deficiency or toxicity are suspected (Bordelon et al, 2009; Aspray et al, 2014; Garg et al., 2018; Sempos et al., 2018).

High-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) is considered as the “gold standard for the measurement of 25(OH)D” (Garg, 2019; Krist et al., 2021). HPLC-UV, competitive protein-binding assays (CPBA) and immunoassays may also be applied in the measurement of 25(OH)D. Approximately seventy percent of 25(OH)D testing is performed using immunoassay-based techniques due to automation, relatively low cost and small sample required to run the test (Garg, 2018). A statement released by the US Preventative Services Task Force in 2021 reported that current evidence showed that results from Vitamin D analysis “vary by testing method and between laboratories using the same testing methods” (Krist et al., 2021).

Binding assays which may be utilised in Vitamin D analyses include CPBA, radioimmunoassay (RIA) and chemiluminescence immunoassay (CLIA). Organic solvent extraction and chromatography are applied prior to performing CPBA. The prime disadvantage of CPBA is the inability to differentiate between 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, leading to underestimation of 25(OH)D at low levels and overestimation of 25(OH)D at high levels. This disadvantage led to the withdrawal of CPBA from the market. The first RIAs were known to utilise small samples and iodine (I<sup>125</sup>) as the radioactive component. They exhibited accurate results, were not costly and not subjected to non-specific interferences. Conversely, they involve use of radionuclides and several of these

methods differentiated between 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>. The basis of CLIA is the “dissociation of 25(OH)D from its binding protein (VDBP)” which is in turn “bound to the specific phase antibody” followed by “the addition of magnetic particles coated with antibody against a 25(OH)D-isolumino tracer”. The unbound fraction is then discarded through a “wash cycle”. A chemiluminescent reaction is initiated through addition of reagents. Relative light units detected by a photomultiplier are “inversely proportional to the concentration of 25(OH)D” (Altieri et al., 2020).

Initial studies of serum Vitamin D levels were carried out using HPLC-UV as it is lacking in sufficient sensitivity for detection of “low levels of 1,25(OH)<sub>2</sub>D<sub>2</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub>” and thus, this procedure is now reserved for research purposes. HPLC-MS/MS is capable of measuring both 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> exhibiting exceptional sensitivity for the measurement of analytes as low as 0.07ng/mL up to 100ng/mL. Disadvantageously, HPLC-MS/MS “has a poor rate of production” and requires an expert analyst. In addition, HPLC-MS/MS can measure several Vitamin D metabolites present within a single sample. The possibility of erroneous results in the presence of Vitamin D<sub>2</sub> and Vitamin D<sub>3</sub> epimers should not be overlooked (Altieri et al., 2020).

Table 1.1 compares the advantages and disadvantages of immunoassay-based techniques with HPLC-MS/MS in the measurement of Vitamin D.

**Table 1.1:** Immunoassay vs HPCL-MS for the measurement of Vitamin D

	<b>Advantages</b>	<b>Disadvantages</b>
<b>Immunoassay</b>	Low cost (Garg, 2018)	May lead to underestimation or overestimation of total 25(OH)D levels (Krist et al., 2021)
	Small sample size (Garg, 2018)	Cannot distinguish between 25(OH)D3 and 25(OH)D2 (Garg, 2018; Altieri et al., 2020)
	Easy to perform and does not require specialised expertise (Garg, 2018)	Lower specificity: Cross-reactivity between Vitamin D and its metabolites (Garg, 2018)
	Automation (Garg, 2018)	Extraction of 25(OH)D may be challenging (Garg, 2018)
<b>HPLC-MS/MS</b>	Increased specificity (Garg, 2018)	High cost (Garg, 2018)
	Ability to differentiate 25(OH)D3, 25(OH)D2 and Vitamin D metabolites (Garg, 2018; Altieri et al., 2020)	Variability and risk of error in results (Garg, 2018; Krist et al., 2021)
	Gold standard (Garg, 2018; Altieri et al., 2020; Krist et al., 2021)	Complicated process performed by experts in the field (Garg, 2018; Krist et al., 2021)
		Do not distinguish between epimers (Garg, 2018)
Time consuming (Altieri et al., 2020)		
	Not readily available (Altieri et al., 2020)	

In 2010, the Vitamin D Standardisation Program (VDSP), “organized by the Office of Dietary Supplements of the National Institutes of Health” (USA), was established “to address [the] well-documented assay variation” amongst Vitamin D testing methodologies. “The standardised laboratory measurement of serum total 25(OH)D” using high-performance liquid chromatography coupled with mass spectrometry is regarded as the “gold-standard reference measurement procedure” (RMP), providing an “accurate” value of Vitamin D, “comparable” to ““true” values for serum total 25(OH)D with stated statistical limits” (Durazo-Arvizu et al., 2017, Rabenberg et al., 2018).

### **1.3 Medical Device Regulation**

The European Union in-vitro diagnostics regulation (Regulation (EU) 2017/746 (EU IVDR)), has entered into application on 26<sup>th</sup> May 2022.<sup>1</sup> This new regulation puts forward an updated risk classification system for in-vitro diagnostic (IVD) medical devices which streamlines the classification of IVD medical devices internationally, as advocated by the Global Harmonization Task Force.<sup>1</sup> The new regulation is more comprehensible and application to new IVD medical devices is facilitated. The majority of IVD medical devices currently on the market will now require “certification by notified bodies”, enhancing patient safety. Devices categorized as Class A devices are self-certified by the manufacturer whereas Class B, Class C and Class D devices require a conformity assessment to be carried out by a Notified Body which subjects manufacturers to “complex requirements and scrutiny” which may in turn lead to certain IVD medical

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<sup>1</sup> European Commission. Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU [Internet]. Official Journal of The European Union. 2017; L117:176-332 [cited 2022 Jun 10]. Available from: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0746>

devices being removed from the EU market.<sup>2</sup> When selecting a medical device for use within a clinical scenario, it is necessary that the healthcare professional making use of the device in practice to ensure that the device, in this case a Vitamin D POCT, is in line with Regulation (EU) 2017/746 (EU IVDR).

#### **1.4 Guidelines for Vitamin D Testing**

Increased awareness of the implication of Vitamin D within multiple disease states and its role as “an immunomodulator hormone” at the peak of the COVID-19 global pandemic has led to an increased demand for Vitamin D testing and Vitamin D supplementation and in turn, increased medical expenditure on healthcare systems (Felcher et al, 2017; Mohan et al., 2020; Patel et al., 2020)

The US Preventative Services Task Force, the American Board of Internal Medicine Foundation, the National Osteoporosis Society (UK) and the Endocrine Society (USA), amongst other associations, do not recommend screening for Vitamin D deficiency in the general population. In an initiative to cut down on unnecessary testing and procedures, the American Board of Internal Medicine Foundation set up the “Choosing Wisely” program which does not support screening for Vitamin D deficiency in patients who are at low risk. NICE (UK) does not recommend routine testing of Vitamin D status unless patients are at risk of developing Vitamin D deficiency e.g. metabolic disorders, exhibit clinical features of Vitamin D deficiency or are about to start medication for the treatment of osteoporosis. The Australian Department of Health have established a set of requesting

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<sup>2</sup> Reinikainen M, Suppo M. Medical Device White Paper Series: Explaining IVD classification issues [Internet]. [cited 2022 Jun 9]. Available from: [https://www.bsigroup.com/globalassets/meddev/localfiles/fr-fr/whitepapers/md-classification\\_issues\\_for\\_ivd.pdf](https://www.bsigroup.com/globalassets/meddev/localfiles/fr-fr/whitepapers/md-classification_issues_for_ivd.pdf)

guidelines which have restricted testing to patients at high risk of developing Vitamin D deficiency. These guidelines include risk factors and symptoms associated with Vitamin D deficiency (Holick et al., 2011; Aspray et al., 2014; Felcher et al., 2017, Bjerg et al., 2019; Krist et al, 2021).

Risk factors for developing Vitamin D deficiency include calcium or parathyroid disorders, malnutrition syndromes, chronic kidney disease, bone disease and those who are on specific medications for example steroids, cholestyramine, anticonvulsants, anti-retrovirals, rifampicin, specific antiepileptics, or certain HIV medications (Aspray et al., 2014; Francis et al., 2015; Felcher et al., 2017; Bjerg et al., 2019).

The Wirral University Teaching Hospital (NHS) and Nottinghamshire Area Prescribing Committee (NHS) consider age extremities (infants and children under 5 years and people over 65), pigmented skin (non-white ethnicity), obesity (BMI > 30), lack of sunlight exposure or use of skin-concealing clothing or strict sunscreen use, conditions related to malabsorption (e.g. inflammatory bowel disease, pancreatic insufficiency, coeliac disease), pregnancy and vegetarianism to be risk factors for developing Vitamin D insufficiency or deficiency (Gorey et al., 2019).<sup>3, 4</sup>

Symptoms associated with Vitamin D deficiency include bone pain or discomfort “without preceding mechanical injury”, fragility fractures, low bone density scores,

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<sup>3</sup>Cowan A, Pugh R, McCaughey A. Treatment of Vitamin D Deficiency in Adults v2 [Internet]. UK, NHS: Wirral Clinical Commissioning Group; 2017 [updated 2020; cited 2022 Jun 10]. Available from: [https://mm.wirral.nhs.uk/document\\_uploads/guidelines/Vitamin%20D%20Guidelines%20for%20Adults%20v2.pdf](https://mm.wirral.nhs.uk/document_uploads/guidelines/Vitamin%20D%20Guidelines%20for%20Adults%20v2.pdf)

<sup>4</sup>Theobald J, Sherwood N, Catt L, Sutton J, Nottingham Osteoporosis Group. Vitamin D Management in Adults V5.0 [Internet]. UK, NHS: Nottinghamshire Area Prescribing Committee; 2021 [cited 2022 Jun 10]. Available from: <https://www.nottsapc.nhs.uk/media/1248/vitamin-d-guidelines-adults.pdf>

rickets, osteomalacia, muscle aches and proximal myopathy. Incidence of carpopedal spasm, seizures, tetany or irritability due to hypocalcaemia require immediate medical attention (Aspray et al., 2014; Bordelon et al., 2009; Bjerg et al., 2019).

Routine monitoring of Vitamin D is recommended in the incidence of “symptomatic Vitamin D deficiency or malabsorption and where poor compliance with medication is suspected”. Vitamin D monitoring is carried out at three to six-month intervals and is not recommended for patients on long term maintenance therapy of daily doses up to 2000IU unless patients develop symptoms suggestive of hypercalcaemia (polyuria, polydipsia, confusion, anorexia, vomiting, muscle weakness) or Vitamin D toxicosis. Patients on antiresorptive therapy for the treatment of osteoporosis should have their Vitamin D levels assessed annually (Aspray et al., 2014; Francis et al., 2015).

### **1.5 Guidelines for Treatment with Vitamin D**

Patients with sufficient levels of Vitamin D should be provided reassurance and counselling on maintaining adequate Vitamin D levels through their diet and safe sunlight exposure (Francis et al., 2015). The human body can produce its own Vitamin D through exposure to UVB rays. During the warmer months (March to October), 10 to 15 minutes of exposure to direct sunlight having forearms, hands and lower limbs exposed, without the application of sunscreen during peak hours of UV sunlight (11am to 3pm), is suggested to maintain adequate Vitamin D levels. Individuals with darker skin will require more time to produce equivalent amounts of Vitamin D. Sunlight during Autumn and Winter contains insufficient UVB wavelength for the skin to produce its own Vitamin D and thus, Vitamin D levels are maintained through Vitamin D stores and consumption

of Vitamin D rich foods which include animal produce such as red meat, dairy products and egg yolk, oily fish, mushrooms, and foods which are fortified with Vitamin D, such as breakfast cereals and some dairy products. Infant formulas are also fortified with Vitamin D (Gorey et al., 2019).

Adequate sunlight exposure and consumption of Vitamin D-rich foods is not sufficient to rectify Vitamin D levels in those who are deficient. Pregnant and lactating mothers, individuals with reduced sunlight exposure and those age 65 or older should ensure adequate Vitamin D consumption at minimum daily doses of 400IU.<sup>3</sup> The Nottinghamshire Area Prescribing Committee (NHS) and National Osteoporosis Society practical clinical guideline on Vitamin D and bone health advises that treatment with Vitamin D supplementation is initiated for patients who have deficient levels of 25(OH)D and patients who have insufficient levels of 25(OH)D and satisfy one or more of the risk factors for Vitamin D (Francis et al., 2015).<sup>4</sup>

When rapid correction of Vitamin D levels is required, treatment with fixed loading doses of oral Vitamin D<sub>3</sub> followed by regular maintenance therapy is recommended. The administration of loading doses is unnecessary in less urgent clinical scenarios or when Vitamin D is prescribed alongside an antiresorptive agent (Aspray et al., 2014). Dosage regimens for the treatment of Vitamin D deficiency using oral Vitamin D<sub>3</sub> supplementation are described in Table 1.2.



**Table 1.2:** Dosing regimen for treatment of Vitamin D deficiency

	<b>Evidence</b>	<b>Cholecalciferol Dose and Regimen</b>	<b>Duration of Treatment</b>
Loading Dose	First Line	40,000 IU weekly	7 weeks
		50,000 IU weekly	6 weeks
	Second Line	3,200 IU daily	12-13 weeks
Maintenance Therapy	First Line	20,000 IU every 4 weeks	Indefinitely
		25,000 IU monthly	
	Second Line	800 IU – 4000IU daily	

Adapted from: Cowan A, Pugh R, McCaughey A. Treatment of Vitamin D Deficiency in Adults v2 [Internet]. UK: Wirral Clinical Commissioning Group: NHS; 2017 [updated 2020 June; cited 2022 May 31]. Available from: [https://mm.wirral.nhs.uk/document\\_uploads/guidelines/Vitamin%20D%20Guidelines%20for%20Adults%20v2.pdf](https://mm.wirral.nhs.uk/document_uploads/guidelines/Vitamin%20D%20Guidelines%20for%20Adults%20v2.pdf)

Vitamin D toxicosis is “characterised by marked hypercalcaemia, hyperphosphatemia and hypercalciuria” alongside levels of 25(OH)D of 100-150ng/mL or above and is a very rare occurrence. Generally, oral supplementation of Vitamin D is rarely associated with the risk of Vitamin D toxicity (Aspray et al., 2014).

Elderly patients are at an increased risk of Vitamin D deficiency owing to a number of factors which include reduced sun exposure and decreased “capacity to generate Vitamin D”. Calcium and Vitamin D supplementation, at recommended daily doses of 1-1.2g and 800IU respectively, are indicated in elderly patients with reduced mobility and frailty, upon recommendation of the Joint Formulary for the Management of Osteoporosis. Intestinal Malabsorption and Chronic Liver Disease warrant administration of Vitamin D in pharmacological doses such as 300,000 IU of ergocalciferol via intramuscular injection at 3-month intervals, as necessary. If levels of serum 25(OH)D are adequate, the dose is skipped and the patient is reassessed 3 months thereafter. Renal patients at end stage

disease are prescribed alfacalcidol upon recommendation of a renal consultant and monitored as necessary. Other CKD patients should be treated as healthy individuals<sup>3</sup>.

## **1.6 The Local Scenario**

Bonnici et al. carried out a study which addressed the knowledge and management of Vitamin D deficiency by Maltese doctors in 2020. The study identified that similar to other countries namely the UK, Australia and Saudi Arabia, Maltese physicians require further comprehension of the management of low levels of Vitamin D. Participant doctors cited the deficient serum 25(OH)D levels (<20ng/mL) in accordance to levels followed at the national hospital which is Mater Dei Hospital (MDH) albeit there being much debate regarding the validity of these cut-off points, as is observed in the international scenario highlighted by Tarn et al. (2016). The level of knowledge of Maltese doctors on Vitamin D is reflected in the management prescribed to their patients presenting with Vitamin D deficiency (Bonnici et al., 2020). Vitamin D testing procedures through the national hospital are restricted to referral by consultant physicians. For general practitioners working outside the general hospital to assess Vitamin D levels for their patients, they must resort to POCT methods or referral to a private hospital or clinic.

## **1.7 Rationale for the Study**

The complexity and expense of running HPLC-MS/MS in the analysis of Vitamin D in a laboratory creates a barrier to Vitamin D testing. The utilisation of immunoassays, which provide specific and sensitive measurements of complex samples facilitates analysis of parameters and is also applicable to ambulatory settings (Matsuda et al., 2015). Coupling

of Vitamin D Point-of-Care Testing with non-invasive screening tools for Vitamin D deficiency obviates “unnecessary supplementation and blood testing”. The development of a Vitamin D POCT framework contributes to the standardisation of service provision of Vitamin D POCT testing holistically, benefitting healthcare facilities, healthcare providers and patients. The ease of use of POCT decreases the load on medical laboratories. The establishment of a set procedure for Vitamin D POCT followed by a guideline facilitates practice, ensuring the provision of a consistent service to patients seeking a rapid and minimally invasive approach in the assessment of Vitamin D levels.

## **1.8 Aims and Objectives**

The aim was to establish a framework for pharmacist-led Vitamin D POCT in primary care.

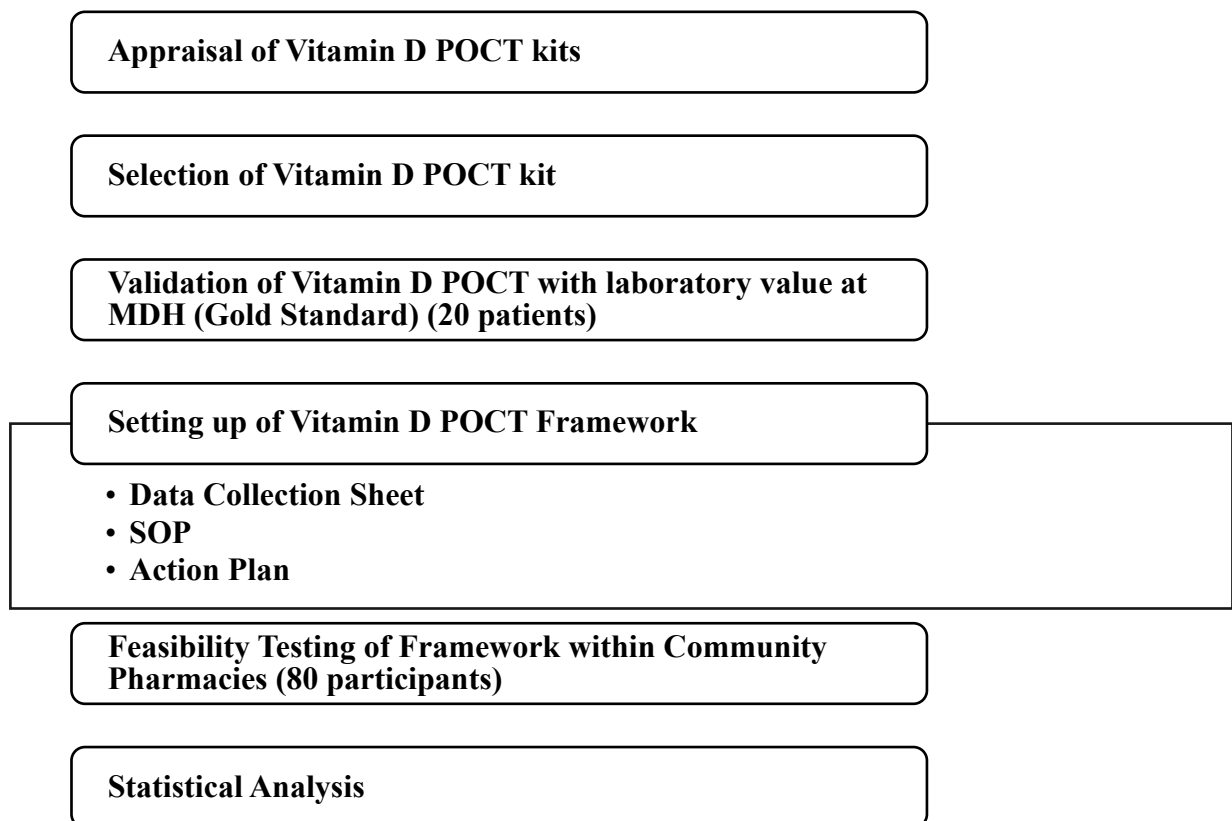
The objectives were to:

- 1) Review available Vitamin D POCT
- 2) Validate Vitamin D POCT versus the gold standard
- 3) Develop and validate a framework for Vitamin D POCT which can be implemented in the local pharmacy care scenario
- 4) Assess feasibility of the pharmacist-led framework within community pharmacy

## **Chapter 2 – Methodology**

## 2.1 Study Design

The flow diagram outlines the study design from appraisal of Vitamin D POCT, selecting the Vitamin D POCT kit for use in the study, validation of the test kit against the laboratory values from MDH (gold standard) setting up the Vitamin D testing framework including a Data Collection Sheet, Standard Operating Procedure (SOP) and an Action Plan, validation of the proposed framework by an expert panel, and testing the feasibility of running the novel pharmacist-led service within a community pharmacy setting. Approval to carry out the study was granted from the University of Malta Faculty of Medicine and Surgery Research Ethics Committee (Ref: UREC-DP2112002MED) (Appendix 1).



**Figure 2.1:** Study Design

## **2.2 Selection and Validation of Vitamin D POCT kit**

The first phase of the study involved review of Vitamin D testing methods and available Vitamin D POCT kits. Comparison of Vitamin D POCT assessed device and manufacturer, technology used, storage and stability, specimen type, testing time, results, test range, quality control, sensitivity and cost. The Vitamin D Rapid Test Cassette (Whole Blood) by Acro Biotech Inc. was selected for use in the study.

The Vitamin D POCT was validated against Vitamin D MDH laboratory values (gold standard) obtained from 20 patients. These patients were attending for routine follow-up testing of their Vitamin D levels and were invited and recruited to the study for POCT by an intermediary. Consent Form, Patient Information Sheet and Recruitment Letter in English or Maltese were provided to each patient at recruitment.

The Vitamin D POCT was carried out within a private consultation room at MDH, following the procedure specified in the developed SOP. POCT and interpretation of the result were carried out by the researcher. Duration for the testing procedure was around 10 minutes. The laboratory result with no other patient information was provided to the researcher by the intermediary for comparison with the POCT result.

## **2.3 Development of Vitamin D POCT Framework**

A Vitamin D Point-of-Care testing framework was set up consisting of a Data Collection Sheet, SOP and an Action Plan. The Data Collection Sheet allows for non-invasive assessment of risk factors for developing Vitamin D deficiency. Development of the Data

Collection Sheet involved review of screening tools which have been developed by other authors (Lukaszuk et al., 2012; Deschasaux et al., 2016; Ferrari and Prosser, 2016; Felcher et al., 2017; Garg et al., 2019; Kahwati et al., 2021) taking into consideration reported limitations and recommendations for improvement, serving as an update to current literature. The Data Collection Sheet was made available in both English and Maltese.

The Data Collection Sheet was set up to be completed by the primary researcher through an interview with the patient. It consists of twenty-four questions, seven of which are subdivided into two or more questions. The first three questions address patient demographics. The following eight questions address the factors of the participant's lifestyle which influence Vitamin D levels, including occurrence of co-morbid conditions. Questions 12 to 17 address previous Vitamin D testing, willingness to have their Vitamin D level tested at the time and patient perception of the implication of Vitamin D levels on general health. The final seven questions are concerned with Vitamin D supplementation, intake of foods rich in Vitamin D and any other medication or supplementation which the patient was taking at the time of the intervention. An appendix listing the amount of alcohol units per measure of alcoholic drink is attached to the Data Collection Sheet to facilitate patient response to question 6b which concerns amounts of alcohol units consumed weekly. The Fitzpatrick skin phototype (Azevedo et al., 2018) and the Godin exercise score (Godin et al., 2011) were included in the Data Collection Sheet as validated tools to assess skin colour and physical activity, respectively.

The package insert for the AcroBiotech INC. Vitamin D Test Cassette (Whole Blood) was used to develop the SOP. The national representative for this product in Malta was

contacted for verification of use of the POCT device. The SOP was developed in accordance with the official template for SOP made available by the Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta.

The Standard Operating Procedure (SOP) (Appendix 3) is a 12-page document which was developed for use in this study. The SOP offers a step-by-step guide for use of the Vitamin D POCT comprising sample collection and preparation, performing the test and interpreting the result. Definitions, responsibilities, health and safety requirements as well as quality control and maintenance are also accounted for. Flow charts for sample collection and preparation as well as for performing the test are included. The SOP is specific to the device selected for use: Acro Biotech Inc. Vitamin D Rapid Test Cassette (Whole Blood). If the framework is adapted to be used in similar studies or by healthcare professionals within varied healthcare settings, an SOP for the specific device being used needs to be drawn up.

Patients were provided with patient-specific advice according to relevant guidelines. This aspect of the framework contributes to the practice of personalised medicine. The action plan is meant to standardise pharmacist recommendations following Vitamin D POCT and to ensure patient referral as necessary. Action taken is dependent on the classification of the patient's Vitamin D level, the occurrence of symptoms of Vitamin D deficiency/insufficiency, medications taken and presence of co-morbid conditions. All patients are provided with lifestyle advice on how to maintain adequate Vitamin D levels through a "Vitamin D Information Leaflet". If referral is required, the patient is referred to a physician, this being either a GP or the specialist who is directly handling the patient. The action plan was developed through reviewal of guidelines and peer-reviewed articles



by the following authors and associations: National Institute for Health and Care Excellence; Nottinghamshire Area Prescribing Committee; Wirral Clinical Commissioning Group; Pan Mersey Area Prescribing Committee; Rockwell et al., 2019; Essig et al., 2020.<sup>3-8</sup> All patients were provided an Information Leaflet regarding Vitamin D which was made available in both English and Maltese language alongside a Referral Note or Result Sheet, as required.

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<sup>5</sup>Pan Mersey Area Prescribing Committee (NHS). Treatment of Vitamin D Deficiency in Adults: Prescribing Guideline V1.11 [Internet]. UK: Midlands and Lancashire Commissioning Support Unit; 2020. [cited 2022 Jun 10] Available from: [https://www.panmerseyapc.nhs.uk/media/2146/vitamind\\_adult.pdf](https://www.panmerseyapc.nhs.uk/media/2146/vitamind_adult.pdf)

<sup>6</sup>National Institute for Health and Care Excellence. NICE Vitamin D: supplement use in specific population groups [Internet]. UK NICE; 2017 [cited 2022 Jun 10]. Available from: <https://www.nice.org.uk/guidance/ph56>

<sup>7</sup>National Institute of Health and Care Excellence. Covid-19 Rapid Guideline: Vitamin D (NG187). Public Health England: Scientific Advisory Commission on Nutrition; 2020 [cited 2022 Jun 10]. Available from: <https://www.nice.org.uk/guidance/ng187/resources/covid19-rapid-guideline-vitamin-d-pdf-66142026720709>

<sup>8</sup>National Institute of Health and Care Excellence (NICE). Vitamin D: Supplement use in specific population groups (PH56). Public Health England: Scientific Advisory Commission on Nutrition; 2014 [updated 2017; cited 2022 Jun 10]. Available from: <https://www.nice.org.uk/guidance/ph56>

## **2.4 Validation of Vitamin D POCT Framework**

Validation of the Data Collection Sheet and Information Leaflet was carried out by an expert panel consisting of a general practitioner, a gastroenterologist, a rheumatology-based hospital pharmacist, an orthopaedic surgeon, an academic pharmacist, a clinical pharmacist, a community pharmacist, and two non-healthcare professionals. The Action Plan was validated by the same panel except the two non-healthcare professionals. The panel was asked to review relevance of content, comprehensibility, readability, and presentation. Any further comments were encouraged.

Overall, all documents presented to the validation panel were very well received and participants commented on the novelty of the pharmacist-led service presented. Both general practitioners remarked that they do not currently have the ability to order a Vitamin D test for their patients from MDH. The panel showed particular interest to the action plan, referring to it as a well-written and clearly portrayed guide to Vitamin D supplementation dosing.

The Data Collection Sheet was previously called a Screening Tool. The change of document title was implemented since, by definition, a screening tool should give or indicate a result. Recommendations by the expert panel for the Information Leaflet were to avoid the terminology “direct sunlight” and to indicate that sun exposure during peak hours of UV in the summer months is not suggested due to associated repercussions such as increased risk of developing melanoma. It was suggested to include a flow-diagram within the Action Plan which will serve as a quick and easy visual guide to healthcare professionals.

## **2.5 Feasibility of Vitamin D POCT Framework (Community Pharmacies)**

The feasibility of carrying out the Vitamin D POCT framework within community pharmacies was assessed. The framework was carried out in private clinics within community pharmacy, as agreed upon with the managing pharmacist. The chosen community pharmacy was visited to ensure the availability of adequate facilities to perform the study. The pharmacy was easily accessible to all patients, including those requiring walking aids, and had adequately lit private clinics with an appropriate desk where patient interviews and POCT were carried out.

Eighty participants were recruited by the managing pharmacist by convenience sampling. Persons over the age of 18 of any gender were eligible for participation in the study. The managing pharmacist set up an appointment for the patient to meet the researcher on a date and time identified by the researcher for the pharmaceutical service to be carried out. Test results obtained were provided to patients through a Result Sheet. A Referral Note was used to communicate results obtained to the physician, together with reason for referral and recommendations as according to the developed Action Plan, as necessary.

## **2.6 Statistical Analysis**

All data collected was inputted into a Microsoft Excel® spreadsheet. Statistical analysis was carried out using IBM SPSS®. The Kappa test was used to assess concordance between the MDH laboratory test (gold standard) and the POCT. The Chi-square test was used to investigate the association between two categorical variables. A p-value less than 0.05 was considered statistically significant.

## **Chapter 3 – Results**

### 3.1 Appraisal of Vitamin D POCT kits

The 7 POCT devices reviewed follow internal quality control procedures, following immunoassay-based technology. Table 3.1 compares the 7 Vitamin D POCT identified in terms of manufacturer, local availability and analytical technique applied. Three of the devices identified are currently available locally. All devices follow chromatographic immunoassay techniques.

**Table 3.1:** POCT kits – Manufacturer, local availability, and technique

Device Name	Manufacturer	Available Locally	Technique
Acro Biotech INC. Vitamin D Rapid Test Cassette	AcroBiotech Inc. USA	Yes	Chromatographic immunoassay
Irishlab	Alpha Pharma Life Science Company	No	
Rapi-D for RapiRead™	Global Diagnostics B Belgium		
Test4D™ Quantitative	NanoSpeed Diagnostics Inc. Canada		
Test 4D™ Semi- Quantitative			
Preventis SmarTest Pro® Vitamin D	Preventis GmbH, Germany	Yes	
Sofia® 1 Quidel®	Quidel Corporation San Diego		

**Table 3.2:** POCT kits – Result, test range and sensitivity

<b>Device Name</b>	<b>Result</b>	<b>Test Range (ng/mL)</b>	<b>Sensitivity (ng/mL)</b>
AcroBiotech INC. Vitamin D Rapid Test Cassette	Semi-quantitative	> 10 10-30 30-100 > 100	4
Irslab	Quantitative	4-100	3
Rapi-D for RapiRead™		3-100	
Test 4D™ Quantitative		3-65	3.1
Test 4D™ Semi- Quantitative		> 15 16-25 > 25	
Preventis SmarTest Pro® Vitamin D	Quantitative	5-100	5
Sofia® 1 Quidel®		10-100	3.1

As described in Table 3.2, test kits identified were of semi-quantitative or quantitative nature having varied testing ranges. Sensitivity ranges from 0.9ng/mL to 5ng/mL (mean: 2.73±1.71).

**Table 3.3:** POCT kits – Specimen and test time

<b>Device Name</b>	<b>Specimen</b>	<b>Testing Time (minutes)</b>
AcroBiotech INC. Vitamin D Rapid Test Cassette	20µL fingerstick whole blood	10
Irislab	30µL whole blood or 20µL serum	15
Rapi-D for RapiRead™	10µL finger-prick capillary blood	20
Test4D™ Quantitative	10µL of fresh blood or 5µL serum blood	10
Test 4D™ Semi- Quantitative		
Preventis SmarTest Pro® Vitamin D	10µL finger-prick capillary blood	20
Sofia® 1 Quidel®	100µL serum blood	10

Test kits utilised serum or whole blood samples in quantities of a minimum of 5µL to 100µL of serum blood. A single test kit may require varied amounts of the different blood types to run the test. Testing time ranges from 10 to 20 minutes (Table 3.3).

**Table 3.4:** POCT kits – Device type and pricing

<b>Device Name</b>	<b>Device Type</b>	<b>Price</b>
AcroBiotech INC. Vitamin D Rapid Test Cassette	Cassette	€6.00 per test Pack of 10 kits
Irishlab	Cube reader	N/A
Rapi-D for RapiRead™	Cube reader	€6.70 per test Pack of 25 kits  €420 additional initial one-time cost for cube reader
Test4D™ Quantitative	Cassette & Cube reader	€733.81 for Test4D™ cube reader and 25 kits
Test 4D™ Semi-Quantitative	Cassette	€11.68 per test Pack of 25 kits
Preventis SmarTest Pro® Vitamin D	Cassette & Smartphone App	€26.20 per test Pack of 20 kits
Sofia® 1 Quidel®	Cube reader	€14.05 per test Pack of 25 kits  €2925 for Sofia®1 System €362.10 for Control Kit (60 runs per kit)

Test kits which involve the use of a cube reader or smartphone application to determine the result are considerably more costly and more complex to perform (Table 3.4). All POCT may be stored at room temperature up to their expiration date.



The Acro Biotech Inc. Vitamin D Rapid Test Cassette was selected for use in the study due to the reasons described in Table 3.5.

**Table 3.5:** Advantages of Acro Biotech Inc. Vitamin D Rapid Test Cassette

<b>Cost</b>	Least costly
<b>Ease of Use</b>	Test does not involve use of a cube reader or mobile app and is the least complicated to use and most applicable to ambulatory care
<b>Conformity</b>	Test is in accordance with Directive 98/79 EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices  Acro Biotech, Inc. Vitamin D Rapid Test Cassette (Whole Blood)  EC Declaration of Conformity attached as Appendix 2
<b>Validity</b>	Data of “In-house Clinical Study Report of Vitamin D Rapid Test” carried out by Acro Biotech, Inc. in 2017 reported 93.8% relative accuracy of the semi-quantitative Vitamin D Rapid Test Cassette compared to the quantitative Vitamin D Test (Rapi-D) (N=97)

### 3.2 Validation of POCT

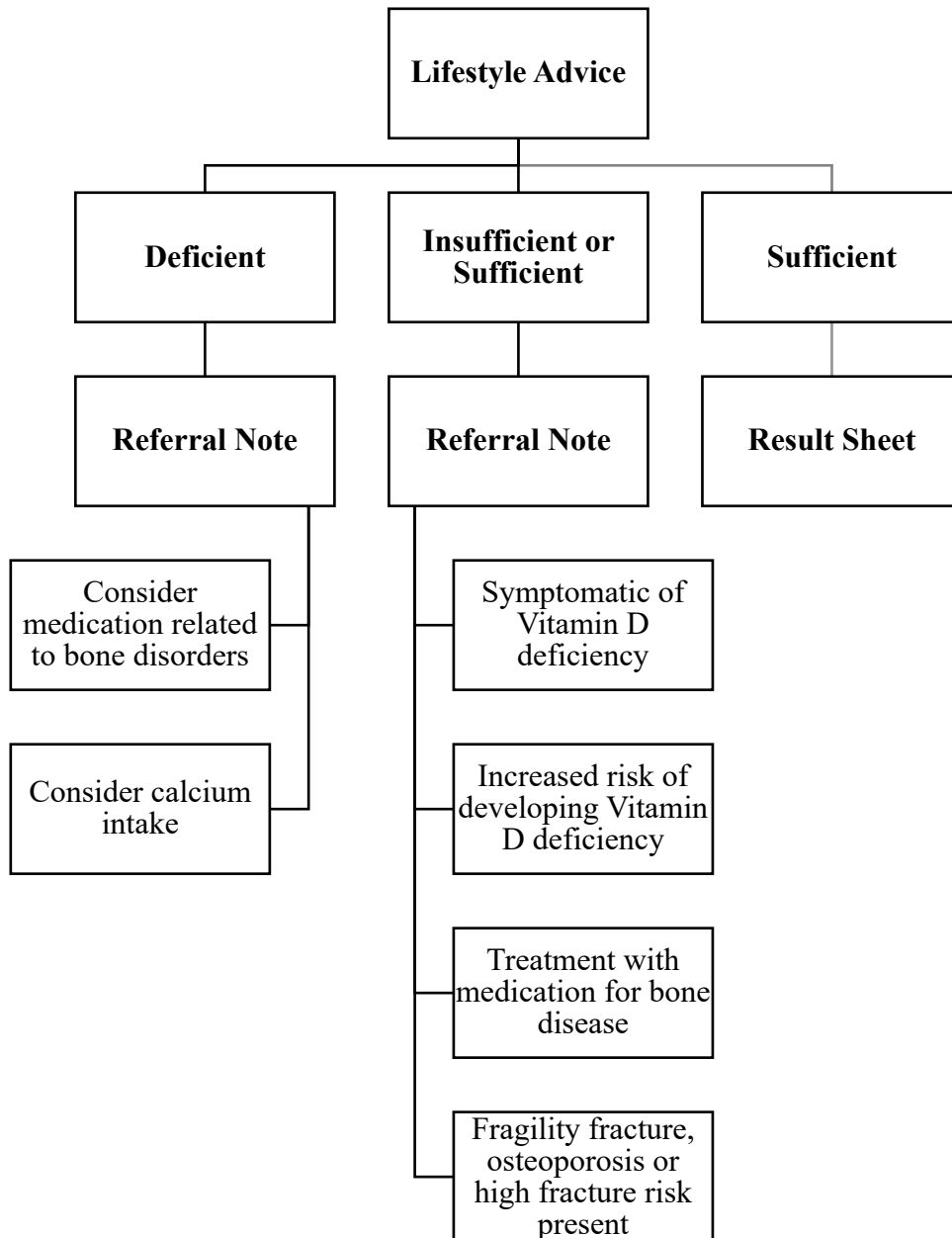
Cohen’s Kappa test (Table 3.6) was used to assess the level of agreement between the Vitamin D level estimated using Acro Biotech Inc. Vitamin D Rapid Test Cassette with the Vitamin D level calculated through serum blood analysis at MDH (gold standard). The Vitamin D levels of 20 participants were assessed using both methodologies. 19 sets of results matched, 1 set of results did not match. The Kappa result obtained was 0.84, which lies in the range from 0.81 to 1 which is interpreted as “almost perfect agreement” (McHugh, 2012). The p-value <0.001 is less than the 0.05 level of significance, implying concordance between the two methods.

**Table 3.6:** Cohen’s Kappa Test – Agreement between Vitamin D testing methods

Measure of Agreement Kappa Value	Standard Error	p-value
0.84	0.158	<0.001

### 3.3 Vitamin D POCT Framework

The validated framework consists of three main components which are a Data Collection Sheet, Standard Operating Procedure and Action Plan (Appendix 3). A flow-chart which simplifies the Action Plan is presented as Figure 3.1.

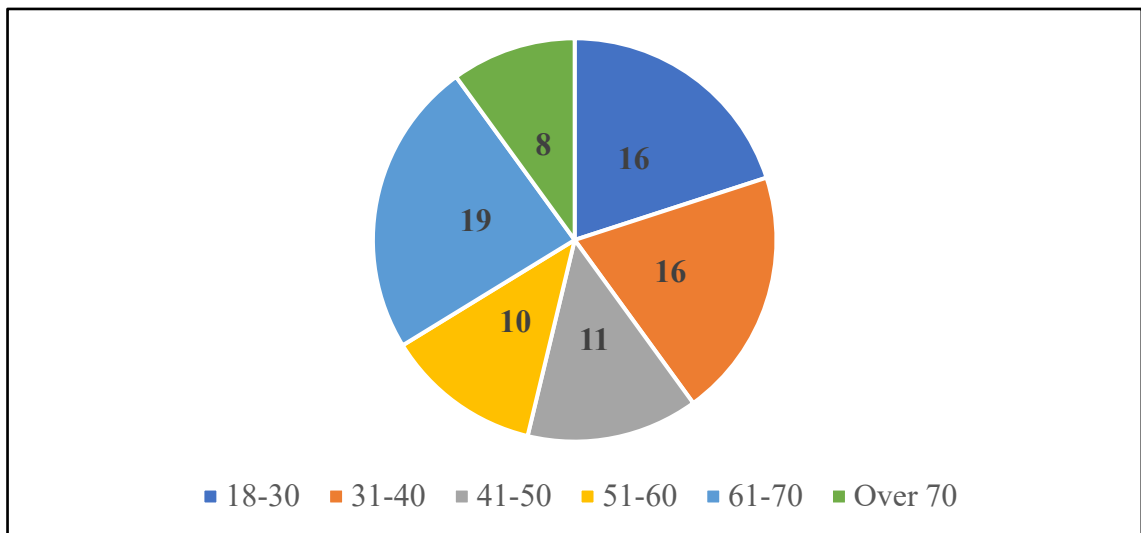


**Figure 3.1:** Flow chart of Action Plan

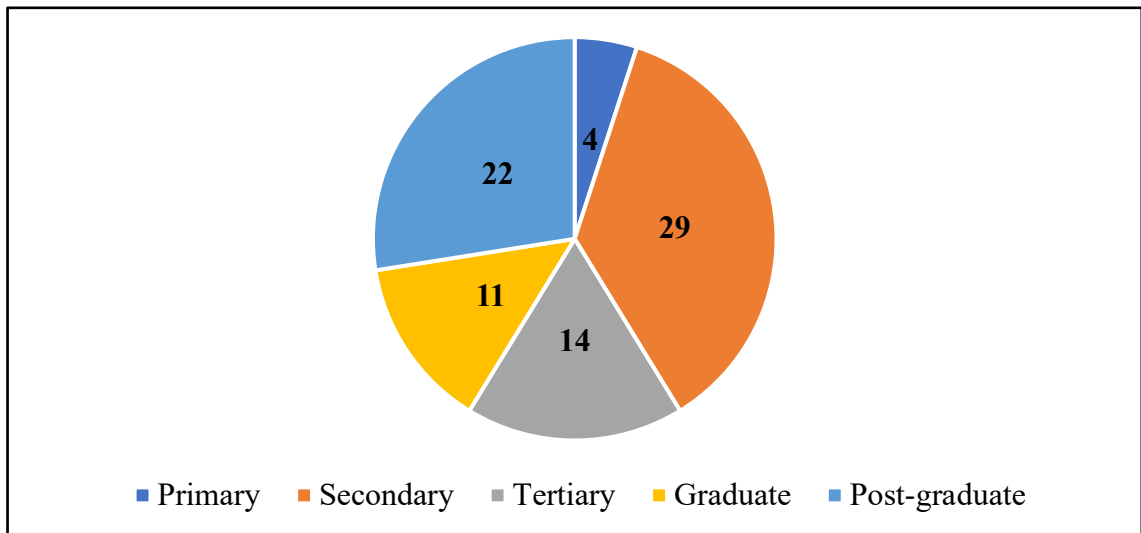
### 3.4 Feasibility of Vitamin D POCT Framework

#### 3.4.1 Demographic Data

Eighty participants were recruited (19 male, 61 female; mean age:  $49.34 \pm 17.67$ , range 22-84 years). Twenty-seven participants were over 60 years of age (Figure 3.2), 29 participants having a secondary level of education (Figure 3.3).

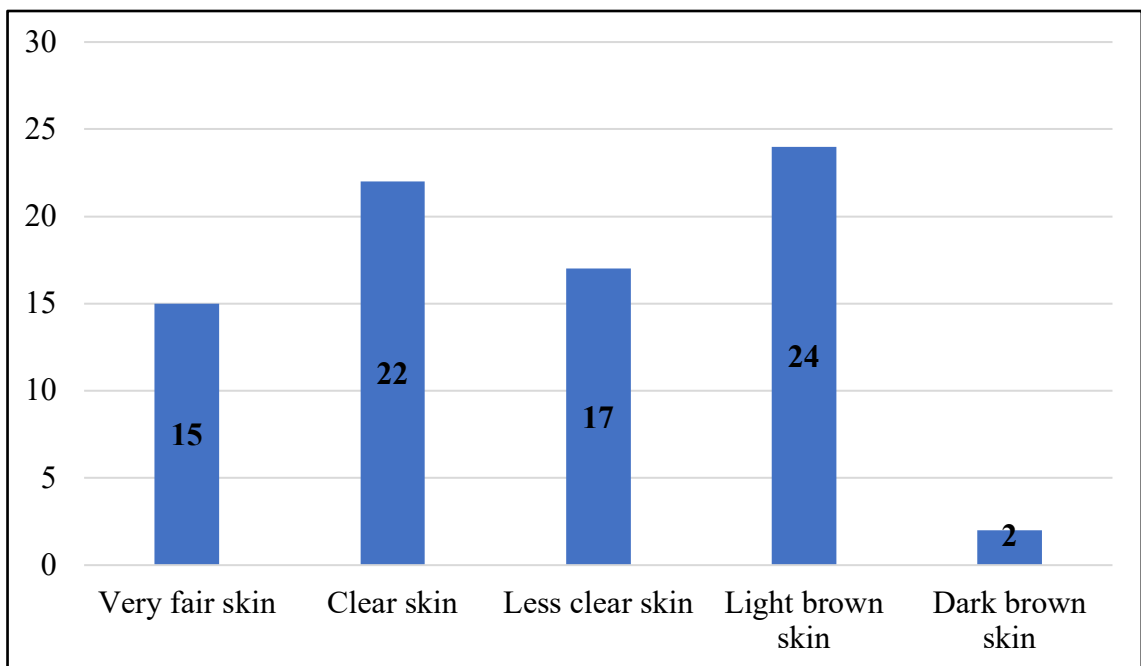


**Figure 3.2:** Age distribution of study population (N=80)



**Figure 3.3:** Level of education of study population (N=80)

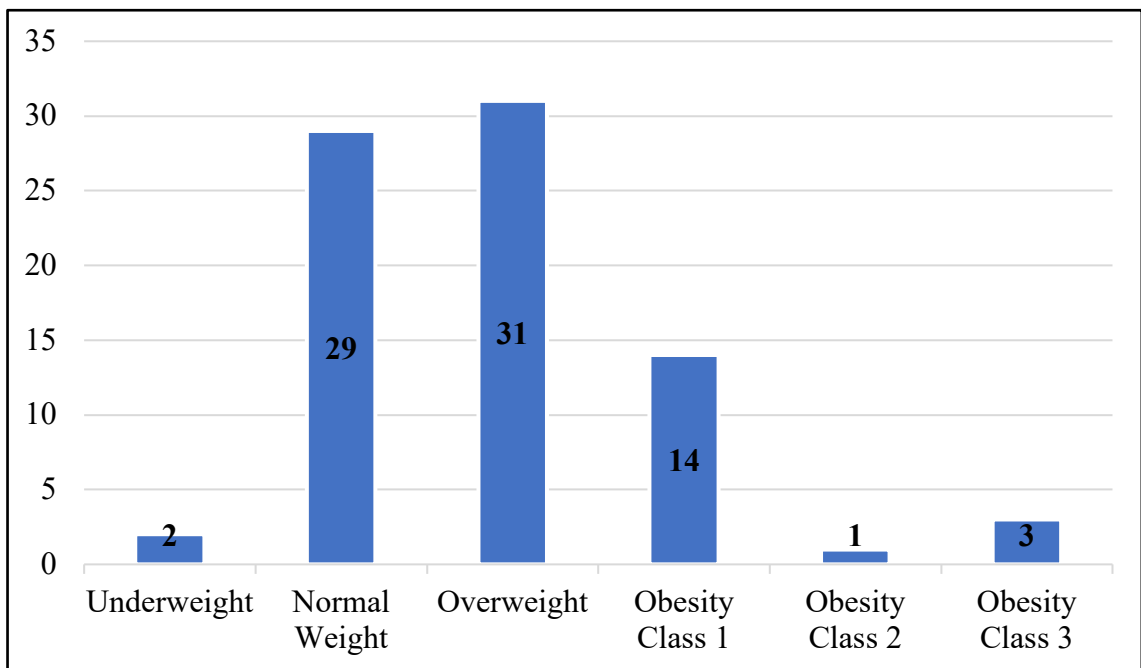
The Fitzpatrick skin phototype was applied to determine the pigment of participants' skin; 26 participants have brown skin types meaning that they rarely/never burn when exposed to the sun (Figure 3.4).



**Figure 3.4:** Fitzpatrick skin phototype of participants (N=80)

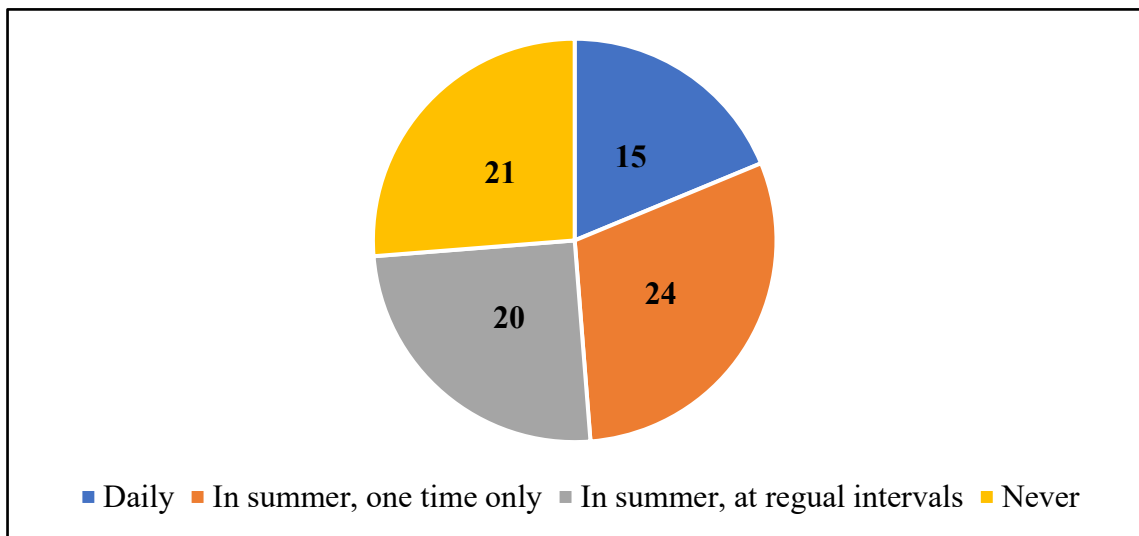
### 3.4.2 Lifestyle choices influencing Vitamin D Levels

Fifty-three participants were insufficiently active, 20 were moderately active and 7 were active, according to the Godin Exercise Score. Forty-nine participants were overweight, 19 of whom were obese, having a BMI of over 30 (Figure 3.5). Twenty participants were current smokers, and 15 participants were previous smokers having stopped smoking at an average of  $17 \pm 12$  years ago. Forty-eight participants consumed alcohol, averaging a weekly consumption of  $8.3 \pm 7.5$  alcohol units.

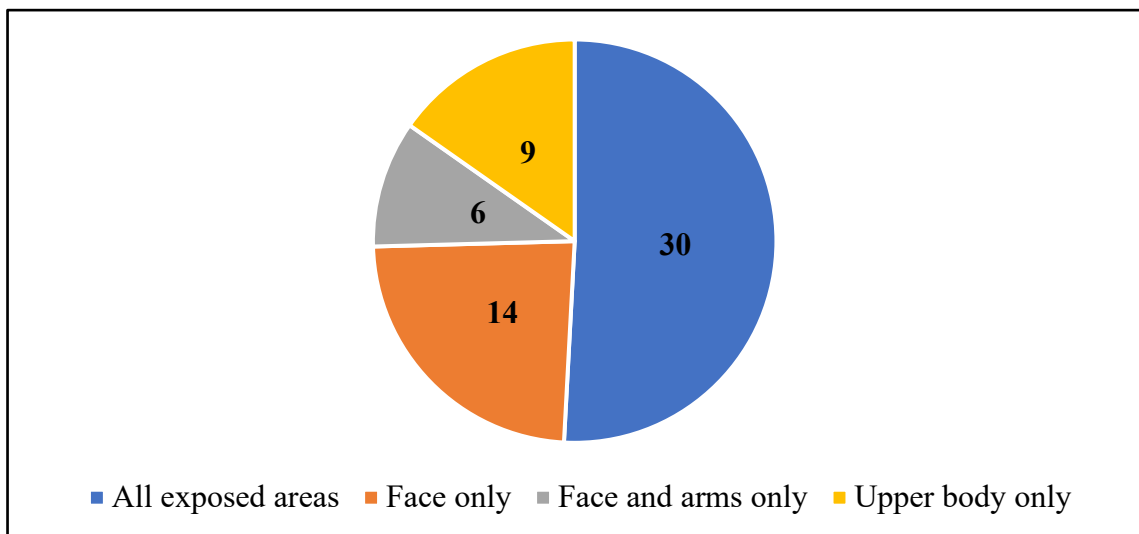


**Figure 3.5:** BMI of study population (N=80)

Sixty-five participants spent less than 30 minutes in the sun daily, 58 having only face and hands exposed. Data collection within community pharmacies was carried out during the winter season is relevant. Most participants (n=24) applied sunblock during the summer months when carrying out outdoor activities, one time only (Figure 3.6). Of the 59 participants who apply sunblock, 30 applied to all exposed areas. Other participants applied only to their face (n=14), face and arms (n=6) or upper body (n=9) (Figure 3.7).



**Figure 3.6:** Sunblock application for participants (N=80)



**Figure 3.7:** Areas of sunblock application (n=59)

Table 3.7 assesses the association between participant perception of the importance of Vitamin D with their daily sun exposure (time in minutes). Participants who are in agreement that Vitamin D is essential for general health are more likely to spend time exposed to direct sunlight ( $p = 0.034$ ).

**Table 3.7:** Importance of Vitamin D vs Sun Exposure (N=80)

			Sun exposure		
			Less than 5 minutes	5-30 minutes	More than 30 minutes
<b>Importance of Vitamin D</b>	Strongly agree	No. of participants	9	24	10
		Percentage	20.9%	55.8%	23.3%
	Agree	No. of participants	5	18	5
		Percentage	17.9%	64.3%	17.9%
	Neutral	No. of participants	6	3	0
		Percentage	66.7%	33.3%	0.0%

$X^2(4) = 10.434, p = 0.034$

### 3.4.3 Medical and Drug History

The presence of a chronic condition for participants was assessed and reported in Table 3.8. Participants were asked to select the group of conditions under which their disorder falls under and specify the condition accordingly. Participants were able to select more than one chronic condition, as necessary. The most cited chronic condition was hypertension. Obesity was calculated by means of BMI calculation where BMI values of 30 or greater were associated with obesity.



**Table 3.8:** Frequency of chronic conditions

<b>Chronic Condition</b>		<b>Frequency</b>
Metabolic Disorder	Obesity	18
	Hypertension	11
	Hypothyroidism	11
	Type 2 diabetes	6
	Hypercholesterolaemia	5
Mental Health Disorder	Anxiety	8
Malignancy	Malignancy	5
Gastro-intestinal Disorder	Crohn's Disease	2
	GORD	1
	Irritable Bowel Syndrome	1
Bone Disorder	Osteoporosis	4
Pain	Back pain	2
	Migraine	2
	Gout	1
Other	Skin disorder	2
	Atrial fibrillation	1
	Genito-urinary disorder	2
	Renal disorder	1

Half of the participants are currently suffering from a metabolic disorder. Metabolic disorders considered included hypertension, hypercholesterolaemia, hypothyroidism, obesity, and type 2 diabetes. Significance is observed in the correlation between the presence of metabolic disorders and insufficient Vitamin D levels.

Table 3.9 shows that 33 of the participants suffering from a metabolic disorder also have insufficient/deficient levels of Vitamin D. A correlation between metabolic disorders and sufficiency of Vitamin D levels was observed ( $p = 0.026$ ).

**Table 3.9:** Vitamin D level vs Presence of metabolic disorder (N=80)

			<b>Metabolic Disorders</b>	
			Yes	No
<b>Sufficient Vitamin D Levels</b>	Yes	No. of participants	7	16
		Percentage	30.4%	69.6%
	No	No. of participants	33	24
		Percentage	57.9%	42.1%

$$X^2(1) = 4.942, p = 0.026$$

Table 3.10 shows that 21 participants that had their Vitamin D levels tested were known to suffer from a chronic condition. There is a positive correlation between the presence of a chronic condition and referral for Vitamin D testing ( $p = 0.042$ ).

**Table 3.10:** Presence of chronic condition/s vs Vitamin D Testing (N=80)

			<b>Vitamin D levels tested previously</b>	
			Yes	No
<b>Presence of chronic condition/s</b>	Yes	No. of participants	21	25
		Percentage	45.7%	54.3%
	No	No. of participants	8	26
		Percentage	23.5%	76.5%

$$X^2(1) = 4.140, p = 0.042$$

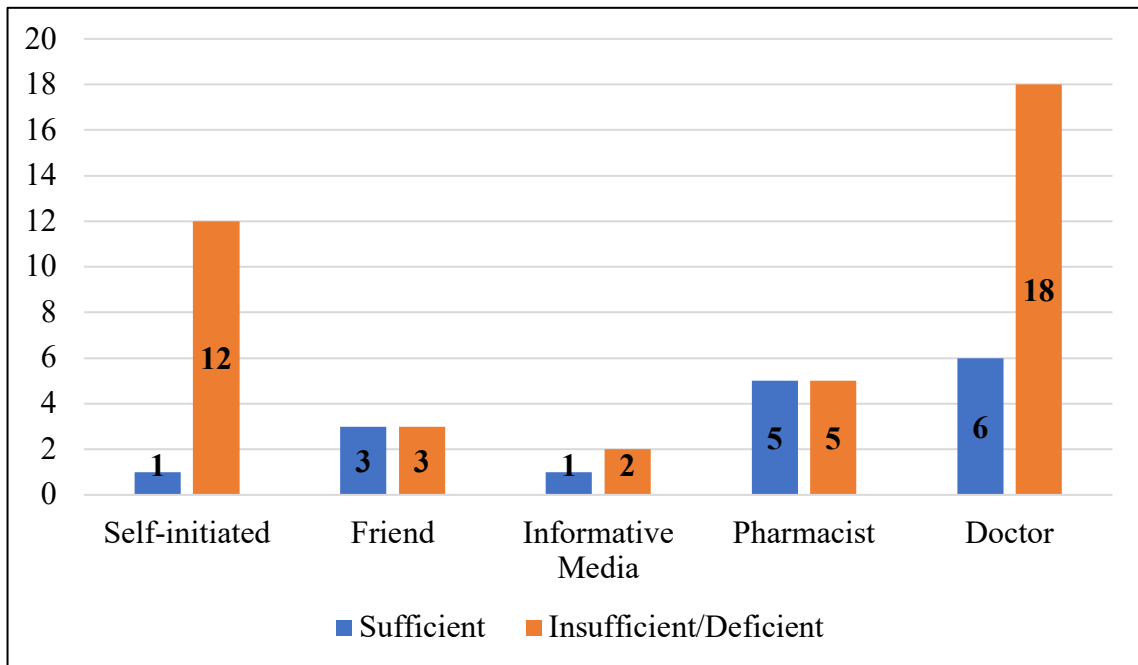
Twenty-seven participants who were referred for Vitamin D testing were referred by their physician, the remaining (n = 2) were referred by a pharmacist. Table 3.11 describes the association between Vitamin D testing and initiation of Vitamin D supplementation. Sixteen participants started Vitamin D supplementation following a Vitamin D test. Initiation of supplementation follows vitamin D testing (p <0.001).

**Table 3.11:** Testing of Vitamin D vs Initiation of Vitamin D supplementation (n=59)

			Initiation of Vitamin D supplementation followed testing	
			Yes	No
Vitamin D levels tested previously	Yes	No. of participants	16	9
		Percentage	64.0%	36.0%
	No	No. of participants	1	33
		Percentage	2.9%	97.1%

$$X^2(1) = 26.186, p = <0.001$$

Figure 3.8 depicts the relationship between participants' Vitamin D status with their recommendation to initiate Vitamin D supplementation. Out of the 56 participants who consumed Vitamin D supplementation, 24 of them were recommended therapy with Vitamin D supplementation by their physician. Despite currently taking Vitamin D supplementation, 18 of the 24 participants have insufficient or deficient levels of Vitamin D.



**Figure 3.8:** Starting supplementation vs Sufficiency of Vitamin D levels (n=56)

Table 3.12 addresses patient adherence to Vitamin D supplementation. Out of the 55 participants who have taken Vitamin D supplementation, 37 participants were still taking Vitamin D supplementation. Once a patient starts Vitamin D he/she is likely to maintain consumption ( $p < 0.001$ )

**Table 3.12:** Adherence to Vitamin D Supplementation (N=80)

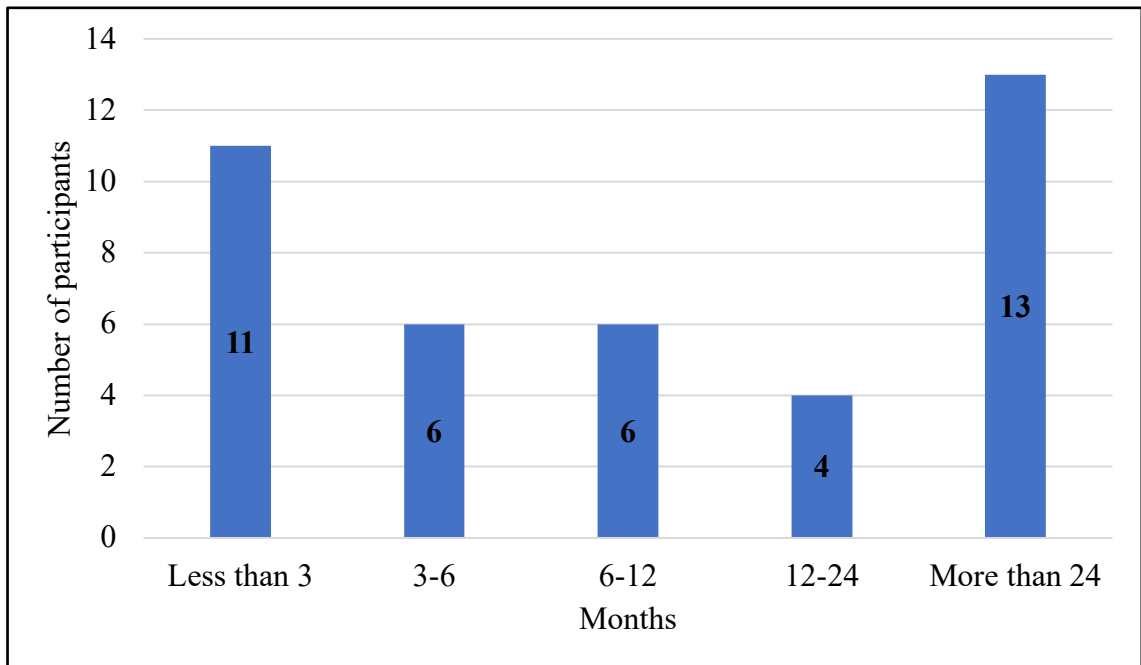
		Current consumption of Vitamin D supplementation		
		Yes	No	
Previous consumption of Vitamin D supplementation	Yes	No. of participants	37	18
		Percentage	67.3%	32.7%
	No	No. of participants	1	24
		Percentage	4.0%	96.0%

$X^2(1) = 27.593, p = < 0.001$

Table 3.13 lists the doses of Vitamin D consumed by patients where the majority of participants who are currently consuming Vitamin D, are taking a dose of 1000 IU of Vitamin D daily (n=40). Thirteen participants who were taking Vitamin D have been doing so for over 2 years, as depicted in Figure 3.9.

**Table 3.13:** Dose of Vitamin D consumed by participants (n=40)

<b>Dosage of Vitamin D Supplement (IU)</b>	<b>Frequency</b>
400	6
600	1
800	1
<b>1000</b>	<b>10</b>
1200	1
1500	2
2000	9
4000	9
8000	1



**Figure 3.9:** Duration of consumption of Vitamin D (n=40)

Table 3.14 depicts the positive correlation between consumption of chronic medication and Vitamin D ( $p < 0.001$ ).

**Table 3.14:** Concurrent consumption of Vitamin D when taking other medication/supplementation (N=80)

			Consumption of other medication/supplementation	
			Yes	No
Current consumption of Vitamin D supplementation	Yes	No. of participants	36	2
		Percentage	94.7%	5.3%
	No	No. of participants	25	17
		Percentage	59.5%	40.5%

$X^2(1) = 13.660, p = < 0.001$

Table 3.15 lists chronic medication and their respective frequency of consumption amongst the 80 participants. Medications cited were grouped in accordance with their indication. The most commonly consumed class of medications was antihypertensives, being taken by approximately 30% (n=19) of participants who consume other medication/supplementation.

**Table 3.15:** Chronic medication/s consumed by participants (n=61)

<b>Medication</b>	<b>Frequency</b>
Antihypertensive*	19
Lipid-lowering agent**	15
Levothyroxine	10
Antidepressant***	9
Oral contraceptive	8
Proton pump inhibitor	8
Magnesium and Vitamin B6	8
Oral hypoglycaemic agent	7
Multivitamin	7
Antiplatelet****	5
Inhaled corticosteroid	4
Short-acting bronchodilator	4
Joint supplementation	4
Fish oil	3
Immunosuppressant*****	3
NSAID	2
Bone supplementation	2
Zinc	2
Other*****	11

\*Antihypertensive includes angiotensin-converting enzyme inhibitor, angiotensin receptor blockers, beta blockers, calcium channel blockers.

\*\*Lipid-lowering agent includes statins and/or fibrates

\*\*\*Antidepressant includes antipsychotics, benzodiazepines and/or SSRI

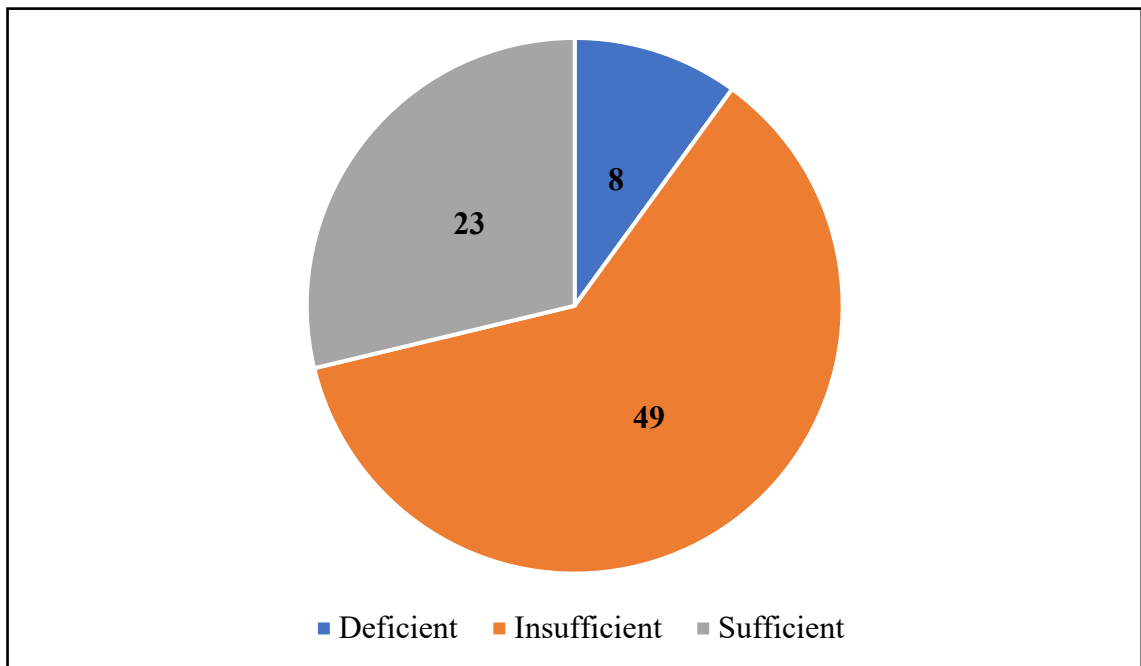
\*\*\*\*Antiplatelet includes aspirin or clopidogrel

\*\*\*\*\*Immunosuppressant includes oral corticosteroids or biologic therapy

\*\*\*\*\*Other includes allopurinol, chemotherapy, iodine supplementation, lactulose, senna, topical oestradiol

### 3.4.4 POCT Results

All testing within community pharmacies was carried out during the winter months. Figure 3.10 depicts the participants' levels of Vitamin D, with the majority (n=49) having insufficient levels. Fifty-seven participants were granted a referral note together with lifestyle advice while the remaining 23 participants were granted a result sheet and lifestyle advice.



**Figure 3.10:** Vitamin D POCT Results (N=80)



## **Chapter 4 – Discussion**

#### **4.1 Vitamin D POCT versus Laboratory Testing**

Commercially available immunoassays and competing binding assays exhibit varied cross-reactivity with diverse Vitamin D metabolites in contrast to chromatographic methods, increasing the risk of bias. The greater part of immunoassays measure total 25(OH)D levels and are unable to distinguish between 25(OH)D<sub>3</sub> and 25(OH)D<sub>2</sub>. Furthermore, complete fidelity in the measurement of 25(OH)D<sub>2</sub> is not persistently present, leading to the underestimation of total 25(OH)D, particularly patients whose main source of Vitamin D is Vitamin D<sub>2</sub>. To date, there is “no analytical technology that combines the high detection capability of LC-MS/MS and the rapid automated properties of immunoassay methods” (Altieri et al., 2020).

An essential aspect of the endorsement of Vitamin D immunoassays lies within the standardisation of measurement of cross-reactants which are resultant to the numerous Vitamin D metabolites found within biological fluids, as reported by Lee et al. (2015). The main pitfall of chromatography-based techniques, especially those which involve mass spectrometry, is due to the occurrence of isotopes (Shah et al., 2018). Optimisation of analytical techniques used in the investigation of Vitamin D levels will consequently filter out overestimation and underestimation of Vitamin D values. Such improvements to the underlying technology of testing procedures may thereafter be scaled to point-of-care devices, rendering such processes more efficacious notwithstanding their feasibility.

The review carried out by Altieri et al. in 2020 analysed the performance of the most applied methods of measurement of 25(OH)D as reported by the DEQAS (the Vitamin D External Quality Assessment Scheme) program from 2014 to 2018. DEQAS (est. 1989)

is the largest specialist External Quality Assessment (EQA) scheme for Vitamin D metabolites in the world. The use of LC-MS/MS in the measurement of 25(OH)D remained consistently high over the years of study, whereas use of immunoassay techniques reportedly decreased by 25%. Use of RIA continues to decline. Inter-laboratory variability was noted to decline for LC-MS/MS methods, with average inaccuracy reported to be below 12%. Conversely, inaccuracies amongst automated immunoassays were highly variable averaging “2.4% to 28.4% at target concentrations between 20 and 40 nmol/L (1 nmol/L = 0.4 ng/mL) and from -5.3% to +20% at target concentrations between 50 and 70 nmol/L”. In light of this, LC-MS/MS continues to be regarded as the “gold standard”, presenting added benefit of the simultaneous measurement of 25(OH)D and its metabolites. DEQAS and other EQA providers consider automated immunoassays with a bias of below 10% as safe for use in clinical practice, with those exceeding the 10% mark to be “rather critical” (Carter et al. 2018; Altieri et al., 2020).

The erratic performance of immunoassays may be attributed to several reasons, including poor antibody specificity, matrix effects, cross-reactivity with other 25(OH)D metabolites and the dissociation of Vitamin D from carrier proteins. The aforementioned limitations do not affect LC-MS/MS methods due to the extraction processes carried out prior to analysis and the high specificity of the analytical technique. Regardless of these limitations, immunoassays and chromatographic analytical methods with “regression slopes close or near to 1.0 with intercepts” render an overall acceptable correlation between automated assays and LC-MS/MD methods (Altieri et al., 2020).

Validation of the AcroBiotech Inc. Vitamin D Rapid Test Cassette whole blood against laboratory values obtained at MDH demonstrated a strong agreement ( $p < 0.001$ ) when the results from the two analytical methods were compared. The EC declaration of conformity for the device is in line with the current IVDR.

#### **4.2 Community Pharmacist Intervention in Vitamin D Deficiency/Insufficiency**

Vitamin D POCT on participants within a community pharmacy setting has shown that 71% ( $n = 57$ ) of participants had insufficient or deficient levels of Vitamin D. This finding is in line with other observational studies from different countries, which have addressed the occurrence of the issue of hypovitaminosis of Vitamin D levels (Holick, 2017; Pludowski et al, 2022).

A positive association was observed between participant perception of the contribution of having sufficient Vitamin D levels towards general health with sun exposure, in terms of time spent exposed to direct sunlight. This finding indicates that participants who are aware that exposure to UVB rays is essential to Vitamin D photosynthesis are more likely to spend time in the sun. This does not at all mean that it is encouraged to spend prolonged periods of time exposed to direct sunlight at times of high UV radiation. It is of detriment to advocate against prolonged UVB exposure which leads to sunburn and DNA damage. Other factors which are known to stimulate Vitamin D dermal photosynthesis include skin pigmentation, age, sunscreen use, season, latitude, and time of day. The aforementioned first four variables were in fact included in the Data Collection Sheet forming part of the proposed framework for Vitamin D POCT. The fact that there are so many constituents which influence dermal Vitamin D photosynthesis poses a challenge

on making a safe and effective recommendation for sun exposure for the general population. Pludowski et al. (2018) comment that the “lack of appreciation that sun exposure is a and ineffective way of obtaining Vitamin D naturally” is often overlooked and is a leading cause of global Vitamin D deficiency.

Vitamin D is a fat-soluble vitamin, mainly stored within adipose tissue. Additional to the role of Vitamin D in calcium homeostasis and bone metabolism, Vitamin D is involved in regulatory processes and development of metabolic disorders. An association was in fact observed between insufficient or deficient levels of Vitamin D and the presence of metabolic disorders, where metabolic disorders considered included hypertension, hypercholesterolaemia, hypotension, obesity, and type 2 diabetes. This finding is in line with the implication of hypovitaminosis of Vitamin D being observed in multiple chronic disease states. In light of this knowledge, an association between the presence of chronic disorders and previous Vitamin D testing has also been noted. Sixteen participants who had their Vitamin D levels tested were subsequently prescribed Vitamin D supplementation (p-value <0.001). Adherence to Vitamin D supplementation was observed through an association between previous consumption of Vitamin D and current consumption of Vitamin D verifying the positive effect of Vitamin D supplementation on quality of life (Hoffman et al., 2015; Rondanelli et al., 2016; Manoy et al., 2017; Heidari et al., 2019; Giustina et al., 2020; Montagnese et al., 2020). The majority of participants who consumed Vitamin D supplementation were instructed to do so by their physician.

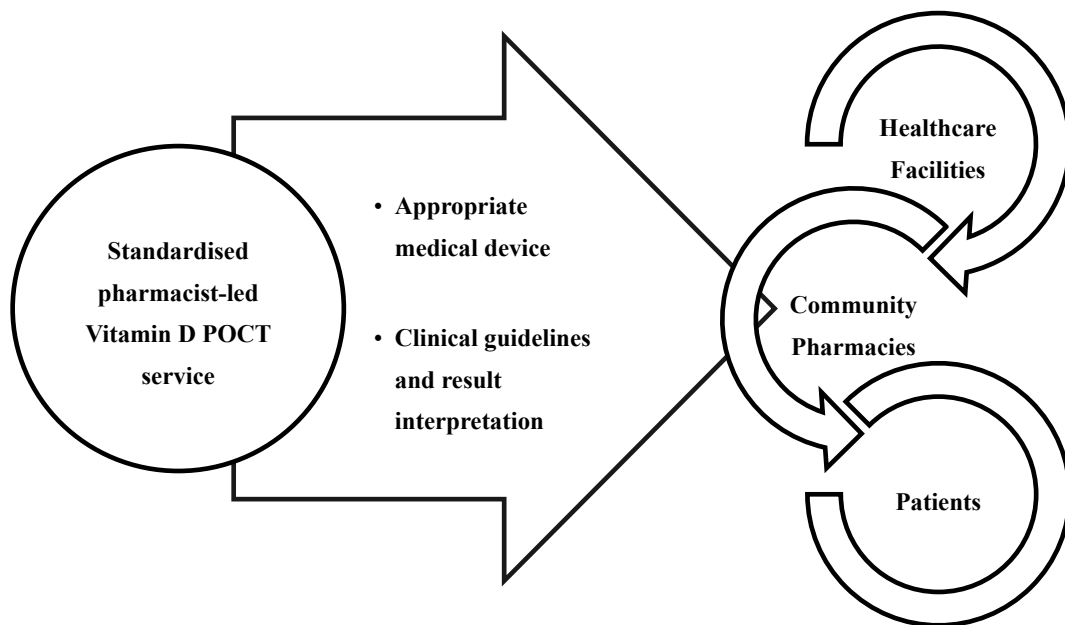
Miao et al. have subsequently stated that Vitamin D supplementation may alleviate the medical burden brought about by metabolic disorders, providing a “new basis for medical therapy” (Cianferotti et al., 2017; Zhang et al., 2019; Miao et al., 2020). Participants who

are taking medication for the treatment of chronic conditions are also likely to consume Vitamin D supplementation; the most frequently reported dose of Vitamin D supplementation being that of 1000IU daily which is within the range of the suggested daily dose of Vitamin D: 800 – 2000 IU orally daily (Francis et al., 2015). Pludowski et al. (2018) suggested that keeping Vitamin D deficiency and insufficiency at bay could imply a “significant reduction in most healthcare costs”.

Despite the fact that thirty-eight participants were taking Vitamin D supplementation, only twenty-three participants from those tested within community pharmacies were found to have sufficient levels of Vitamin D and no association between the two variables was found when statistical analysis was carried out. This may be attributed to underdosing of Vitamin D supplementation, short-duration of consumption of the required dose of Vitamin D and/or issues associated with Vitamin D malabsorption. Pludowski et al. (2018) also mentions that the fat-soluble nature of Vitamin D may lead clinicians and other healthcare professionals to underdose Vitamin D due to fear of reaching toxicity. Their study also highlighted the fact that very limited evidence on the health benefits of Vitamin D has emerged from randomised control trials (RCTs). The lack of evidence supporting Vitamin D supplementation from RCTs may be attributed to the fact that trial data was “primarily derived from pharmaceutical drug studies” rather than nutrient-specific studies. Pharmaceutical drug studies falsely assume that nutrient provision includes solely what is provided as part of the study, following a “linear dose-response relation”, which is not the case with Vitamin D (Pludowski et al., 2018).

### 4.3 Significance of the Study

The research has shown that the Maltese population has a tendency towards lower levels of Vitamin D, 71% of the test population having insufficient or deficient levels (<30 ng/ml), mirroring the global scenario. Access to laboratory Vitamin D testing is restricted and requires expert personnel and expensive equipment for analysis using the gold standard method. These factors identified a need for the development of a standardised pharmacist-led Vitamin D POCT service using an appropriate medical device alongside the application of clinical guidelines implemented prior and following testing. This innovative pharmacist-led approach to Vitamin D testing reduces economic burden on healthcare facilities, adds value to clinical pharmacy provision in primary care and benefits patients through harmonisation of Vitamin D analysis, coupled with the appraisal of identification of risks and recommended personalised action plan.



**Figure 4.1:** Vitamin D POCT: A pharmacist-led approach

#### **4.4 Strengths and Limitations**

The proposed framework for Vitamin D POCT has the potential for early screening, especially in primary health care facilities in resource limited settings (Kahwati et al., 2021). The innovation of the study is that, to the knowledge of the researcher, this is the first framework for Vitamin D POCT in a community pharmacy setting comprising of a Data Collection Sheet, Standard Operating Procedure and Action Plan set up to date. This research is impactful in that paves the way to a more economical and seamless healthcare system. The implementation of the framework facilitates access to testing, early detection of Vitamin D deficiency and insufficiency, and patient referral for guided treatment modalities which may be applied by prescribers. The Action Plan contributes to the expertise of prescribers and to the knowledge of their patients. Prescribers benefit from gaining confidence in the field of Vitamin D management. Bonnici et al. have assessed the confidence of Maltese doctors with regards to their knowledge and management of Vitamin D, observing that there is concordance between comprehension of the implications of having deficient/insufficient Vitamin D and their confidence to manage the condition (Bonnici et al., 2020).

The determination of the level of sufficiency of Vitamin D when using the Acro Biotech Inc. Vitamin D Rapid Test Cassette is based on a chromogenic colour indicator which is subject to the opinion of the professional running the analytical test. The researcher ensured that an adequate amount of sunlight was available to compare the intensity of the test line on the test cassette with the colour card provided, at the time stipulated by the instructions for the test kit.



The data obtained through use of the Data Collection Sheet was dependent on patient recall. The lack of an electronic health system which is accessible to all healthcare professionals, as necessary, results in a lot of gaps when assessing patient history. Participants may be inclined to underestimate alcohol intake and cigarette smoking, as well as overestimate healthy eating habits and exercise. A larger sample size has the potential to even out such discrepancies.

Incongruity between observational studies may be attributed to disparities between cut-off points to define Vitamin D deficiency, insufficiency, sufficiency, and toxicity as defined by different associations. Additionally, population characteristics associated with lower Vitamin D levels may necessitate a variation in the Vitamin D requirements of that population (Krist et al., 2021). While the notion that Vitamin D sufficiency and its effect on health-related quality of life has been accepted, the establishment of a set of “official guidelines and benchmarks” is still a challenge. “Intra-assay variation and inter-assay variability” of Vitamin D testing methods is attributable to the lack of standardisation and vast array of analytical techniques for the assessment of Vitamin D available.

#### **4.5 Recommendations for Further Research**

Implementing a scoring system to the Data Collection Sheet may render this instrument into a valuable and inexpensive Screening Tool or taking it a step further, Clinical Decision Support (CDS) tools within Electronic Health Records. The Screening Tool has the potential to deliver superior healthcare to patients whilst simultaneously reducing costs for the healthcare system and/or the patient through regulation of medical procedure orders; reducing workload on the healthcare system due to the technique required to run

LC/MS-MS chromatography which must be carried out by scientists of a certain calibre; and furthermore, reducing risk for the patient. A similar screening tool to the one proposed in this study was developed by De Giuseppe et al in 2022 in Italy. The developed screening tool ‘EVIDENCE-Q’ aims to reduce healthcare costs at different strata of healthcare and has the potential to identify risks for hypovitaminosis of Vitamin D levels at early stages, preventing vitamin D deficiency and avoiding ‘unwarranted supplementation’ (De Giuseppe et al., 2022). This approach resonates with the triple aim proposed by the Institute of Healthcare Improvement “of increasing quality, increasing patient-centred care, and decreasing cost”<sup>9</sup> as well as with the American Board of Internal Medicine’s “Choosing Wisely” initiative which seeks to curtail healthcare services of low impact and superfluous costs<sup>10</sup> (Felcher et al., 2017; Patel et al., 2020).

Further studies being carried out over longer periods of time may overcome both the issue of sample size as well as be able to compare prevalence of Vitamin D deficiency/insufficiency over different times of the year when sun exposure and UV levels are varied.

Further recommendation to studies performed over longer time periods would be to follow-up with patients who were prescribed Vitamin D supplementation by their physician. It is suggested that the researcher assesses whether the physician prescribed a Vitamin D dose in accordance with the advice provided by the researcher following obtaining an insufficient/deficient result for the patient’s Vitamin D level. Furthermore,

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<sup>9</sup>Institute for Healthcare Improvement. Triple Aim Measures [Internet] [cited 2022 May 31]. Available from: <http://www.ihl.org/Engage/Initiatives/TripleAim/Pages/default.aspx>.

<sup>10</sup>American Board of Internal Medicine. Choosing Wisely [Internet]. 2015 [cited 2022 May 31] Available from: <https://www.choosingwisely.org>.

re-testing of Vitamin D levels at an interval following administration of Vitamin D in these patients to assess the effect of supplementation on their Vitamin D status should also be considered.

The requirement for a global consensus regarding the interpretation of Vitamin D values has been recognised by a multitude of authors and organisations (Tsuprykov et al., 2018; Altieri et al., 2020; Amrein et al., 2020; Bonnici et al., 2020). Achieving the aforementioned cut-off points, determining insufficiency and deficiency will shed light on the respective treatment requirements by Vitamin D supplementation through the development of guidelines (Amrein et al., 2020; Bonnici et al., 2020). Identification of these parameters will in turn form an essential component of the validation of Vitamin D analysis both within laboratories and through the use of POCT devices. Efforts towards the standardisation of 25-hydroxyVitamin D assays have been underway for over a decade, namely through the Vitamin D Standardisation Program (VDSP) (Bjerg et al., 2019).

An essential aspect of the endorsement of Vitamin D immunoassays lies within the standardisation of measurement of cross-reactants which are resultant to the multitude of Vitamin D metabolites found within biological fluids. The main pitfall of chromatography-based techniques, especially those which involve mass spectrometry, is due to the occurrence of isotopes (Shah et al., 2018). Optimisation of such analytical techniques and others used in the investigation of Vitamin D levels will consequently filter out over- and underestimation of Vitamin D values. Such improvements to the underlying technology of testing procedures will hopefully be mirrored within smaller

scale point-of-care devices, rendering such processes more efficacious notwithstanding their feasibility.

Conversely, Bjerg et al. (2019) added that standardisation of 25(OH)D assays will not shed light on the perplexity between studies which discuss what levels of 25(OH)D constitute deficiency, insufficiency, sufficiency, and toxicity. Evidence-based unanimity of values which are associated with Vitamin D status is not directly linked to the standardization of Vitamin D testing methods and therefore, further research is also required in this field.

#### **4.6 Conclusion**

The developed framework has the potential to contribute to reducing burden on healthcare facilities, facilitate provision of a pharmacist-led service and access to patients to a reliable and efficient Vitamin D point-of-care testing service. The review identified a POCT that could be used within a framework for community pharmacist-led assessment of Vitamin D within the context of collaborative care. A combination of good sensitivity and high specificity is essential for screening devices. Healthcare professionals making use of POCT as diagnostic tools have the responsibility to ensure that the in-vitro diagnostic device is in line with governing regulation, in this case Regulation (EU) 2017/746, safeguarding patients from erroneous results. Ensuring the validity of POCT adds robustness to the test result obtained. Comparison of POCT results with traditional laboratory test results, which are considered as the gold standard, is a means of validation of POCT and other in-vitro diagnostic medical devices (Wang et al., 2018).

Early detection of Vitamin D deficiency will allow for management to prevent and delay progression of hypovitaminosis of Vitamin D. The development of a Vitamin D POCT framework will lead to the standardisation of the pharmacist-led service provision of Vitamin D POCT testing holistically, benefitting healthcare facilities, healthcare providers and patients. The ease of use of POCT decreases the load on medical laboratories and facilitates access to Vitamin D testing, enabling early detection of insufficiency. The establishment of a set procedure for Vitamin D POCT followed by a guideline will facilitate practice, ensuring the provision of a consistent service to patients seeking a rapid and minimally invasive approach in the assessment of Vitamin D levels.

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## **Appendices**

## Appendix 1: Ethics Approval



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Ref No: 9745\_22092021

2 March 2022

Ms Catherine Anne Busuttil

'Camelot'  
Hope Street,  
MST1301,  
Mosta,  
Malta

With reference to your application submitted to the Faculty Research Ethics Committee in connection with your research entitled:

#### **Vitamin D Point-of-Care Testing**

The Faculty Research Ethics Committee is granting ethical approval for the above-mentioned application.

A handwritten signature in black ink, appearing to read 'Anthony Serracino Inglott'.

Professor Anthony Serracino Inglott  
Chair  
Faculty Research Ethics Committee



## Appendix 2: Acro Biotech, Inc. Vitamin D Rapid Test Cassette (Whole Blood) EC

### Declaration of Conformity



#### *EC Declaration of Conformity*

Manufacturer:

Name: Acro Biotech, Inc.

Address: 9500, 7th str., Unit M, Rancho Cucamonga, CA 91730, USA

European Representative:

Name: MedNet GmbH

Address: Borkstrasse 10, 48163 Muenster, Germany

Product Name: Vitamin D Rapid Test (Whole blood)

Model: Cassette

Classification: Other Device of IVDD 98/79/EC

Conformity Assessment Route: IVDD 98/79/EC Annex III (excluding point 6)

EDMA Code: 12 07 02 90 00

We herewith declare that the above mentioned products meet the transposition into national law, the provisions of the following EC Council Directives and Standards. All supporting documentations are retained under the premises of the manufacturer.

#### DIRECTIVES

General applicable directives:

DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 1998 on in vitro diagnostic medical devices

Standard Applied: EN ISO 13485:2016, EN ISO 14971:2012, EN 13975:2003, EN ISO 18113-1:2011, EN ISO 18113-2:2011, EN 13612:2002/AC:2002, EN ISO 17511:2003, EN ISO 23640:2015, EN 13641:2002, EN ISO 15223-1:2016

Place, Date of Issue: in Rancho Cucamonga on 21/03/2019

Signature: 

Name: Joseph Fan

Position: President

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## Appendix 3: Vitamin D Point-Of-Care Testing Framework (English & Maltese)

### Data Collection Sheet (English)

**Patient Study Number:** \_\_\_\_\_ **Date:** \_\_\_\_\_

*for internal use*

*To be filled in by the primary researcher through an interview with the patient*

#### 1. Age (years)

- 18 – 30
- 31 – 40
- 41 – 50
- 51 – 60
- 61 – 70
- >70

#### 2. Sex

- Male
- Female
- X
- Prefer not to say

#### 3. What is your level of education?

- Primary
- Secondary
- Post-secondary
- Graduate
- Postgraduat

#### 4. Body Mass Index

Weight (kg)	Height (m)	BMI = kg/m <sup>2</sup>

Classification	BMI Category (kg/m <sup>2</sup> )
Underweight	< 18.5
Normal Weight	18.5 – 24.9
Overweight	25.0 – 29.9
Obese Class I	30.0 – 34.9
Obese Class II	35.0 – 39.9
Obese Class III	≥ 40

#### 5. Smoking

**a) What is your smoking status?**

- Current smoker (go to Q5b)
- Previous smoker

Specify how long since you stopped smoking:

\_\_\_\_\_

- Never smoked (go to Q6)

**b) If current smoker, how many cigarettes do you smoke?**

- 1-5 cigarettes daily
- 6-10 cigarettes daily
- 11-20 cigarettes daily
- 1-2 packets of cigarettes daily
- Occasional smoker
- Other

Specify: \_\_\_\_\_

## 6. Alcohol Consumption

### a) Do you drink alcohol?

- Yes (go to Q6b)
- Never consumed alcohol (go to Q7)
- No

### b) If yes, what is your average weekly consumption of alcohol in alcohol units (see appendix)?

---

## 7. Physical Activity

During a typical **7-day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

	Times per Week
<b>Strenuous Exercise</b> – Heart Beats Rapidly Examples: Running, jogging, hockey, football, squash, basketball, judo, vigorous swimming, vigorous long-distance bicycling	_____
<b>Moderate Exercise</b> – Not Exhausting Examples: Fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, dancing	_____
<b>Mild Exercise</b> – Minimal Effort Examples: Yoga, archery, fishing, bowling, golf (without using a cart), easy walking	_____

Adapted from: Godin G. The Godin-Shephard Leisure-Time Physical Activity Questionnaire. Health and Fitness Journal of Canada. 2011;4(1):18-22.

## 8. Sun Exposure

### a) How much time do you spend in the sun daily?

- Less than 5 minutes
- 5 – 30 minutes
- More than 30 minutes

### b) Amount of exposed skin surface

- Face and hands
- Face, hands and arms
- Face, hands and legs
- Face, hands, arms and legs

## 9. Fitzpatrick skin phototype

Typical features	Tanning ability	Skin type	
Very fair skin	Always burns, does not tan	I	
Clear skin	Always burns, sometimes tans	II	
Less clear skin	Sometimes burns, always tans	III	
Light brown skin	Rarely burns, always tans	IV	
Dark brown skin	Never burns, always tans	V	
Black skin	Never burns, always tans	VI	

Adopted from: Azevedo M, Bandeira L, Luza C, Lemos A, Bandeira F. Vitamin D Deficiency, Skin Phototype, Sun Index, and Metabolic Risk Among Patients with High Rates of Sun Exposure Living in the Tropics. *J Clin Aesthet Dermatol.* 2018;11(8):15-18.

## 10. Sunscreen Use

### a) How often do you apply sunblock?

- Daily
- In the summer months when carrying out outdoor activities, one time only
- In the summer months when carrying out outdoor activities, at regular intervals as suggested according to the product leaflet
- Never
- Other

Specify: \_\_\_\_\_

### b) Do you apply sunscreen to all exposed areas?

- Yes
- No

### c) If no, where do you apply sunscreen?

- Face
- Face and arms
- Arms
- Other

Specify: \_\_\_\_\_

## 11. Co-morbidities

- Autoimmune disease

Specify: \_\_\_\_\_

- Cardiovascular disease

Specify: \_\_\_\_\_

- Chronic liver disease

- Cognitive disease

Specify: \_\_\_\_\_

- Diabetes

Specify type: \_\_\_\_\_

- Gastrointestinal

Specify: \_\_\_\_\_

- Infectious disease

Specify: \_\_\_\_\_

- Malignancy

- Metabolic syndrome

Specify: \_\_\_\_\_

- Osteoporosis

Specify history of falls or fractures: \_\_\_\_\_

- Renal disease

- Mental Health Disorder

Specify: \_\_\_\_\_

- Skin conditions

Specify: \_\_\_\_\_

- Pain

Specify: \_\_\_\_\_

**12. Have you ever had your Vitamin D levels tested?**

- Yes (go to Q13)

- No (go to Q15)

**13. Who referred you for Vitamin D testing?**

- Doctor

- Pharmacist

- Other

Specify: \_\_\_\_\_

**14. How was your blood sample analysed?**

- Laboratory analysis
- Using a point-of-care device

**15. Are you interested in having your Vitamin D levels tested?**

- Yes (go to Q16)
- No (go to Q17)

**16. Season of fingerstick blood sample collection**

- Spring
- Summer
- Autumn
- Winter

**17. Do you think having appropriate Vitamin D levels is essential for general health?**

- Strongly agree
- Agree
- Neutral
- Disagree
- Strongly Disagree

**18. Have you ever taken any form of Vitamin D supplementation?**

- Yes (go to Q19)
- No (go to Q21)

**19. Who advised you to take Vitamin D supplementation?**

- Self-initiated
- A friend
- Informative media
- Pharmacist
- Doctor
- Other

Specify: \_\_\_\_\_



**20. Were you advised to take Vitamin D supplementation following a blood test?**

- Yes
- No

**21. To your knowledge, your current Vitamin D levels are**

- Deficient (< 10 ng/mL)
- Insufficient (10 – 30 ng/mL)
- Sufficient (> 30 ng/mL)
- Toxic (>100 ng/mL)
- Don't know

**22. Food rich in Vitamin D**

**a) How often do you consume foods rich in Vitamin D (e.g. eggs, red meat, fish, mushrooms, ricotta cheese)?**

- Daily
- < 3 times weekly
- No consumption

**b) Do you seek foods which are fortified with Vitamin D?**

- Yes
- No

**23. Vitamin D Supplementation**

**a) Are you currently taking any form of Vitamin D supplementation?**

- Yes
- No (go to Q24)

**b) What dosage of Vitamin D supplementation are you taking?**

- Dairy products fortified with Vitamin D
- 400IU daily
- 1000IU daily
- 2000IU daily
- 3000IU daily
- 4000IU daily
- 5000IU daily
- 10,000IU weekly
- 50,000IU weekly
- Other: \_\_\_\_\_

**c) For how long have you been taking the above stated dose?**

- < 3 months
- 3-6 months
- 6-12 months
- 12-24 months
- > 24 months

**24. Medication**

**a) Are you currently taking any medications or other supplementation?**

- Yes
- No

**b) If yes, please specify**

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## Appendix

### Number of alcohol units per measure of alcoholic drink

Type of drink	Number of alcohol units
Single small shot of spirits* (25ml, ABV 40%)	1 unit
Alcopop (275ml, ABV 5.5%)	1.5 units
Small glass of red/white/rose wine (125ml, ABV 12%)	1.5 units
Bottle of lager/beer/cider (330ml, ABV 5%)	1.7 units
Can of lager/beer/cider (440ml, ABV 5%)	2 units
Pint of lower-strength lager/beer/cider (ABV 3.6%)	2 units
Standard glass of red/white/rose wine (175ml, ABV 12%)	2.1 units
Pint of higher-strength lager/beer/cider (ABV 5.2%)	3 units
Large glass of red/white/rose wine (250ml, ABV 12%)	3 units

\*Gin, rum, vodka, whisky, tequila, sambuca. Large (35ml) single measures of spirits are 1.4 units

nhs.co.uk [Internet]. Alcohol units: Alcohol support. c2018-c2021 [cited 2021 Aug 16] Available from: <https://www.nhs.uk/live-well/alcohol-support/calculating-alcohol-units/>

## Formola tal-Ġbir tad-Data

Numru ta' Studju tal-Pazjent : \_\_\_\_\_

Data: \_\_\_\_\_

*għall-użu intern*

*Għandha timentela mir-riċerkatur permezz ta' intervista mal-pazjent*

### 25. Età (snin)

- 18 – 30
- 31 – 40
- 41 – 50
- 51 – 60
- 61 – 70
- >70

### 26. Sess

- Raġel
- Mara
- X
- Nippreferi ma ngħidx

### 27. X'inhu l-livell ta' edukazzjoni tieghek?

- Primarja
- Sekondarja
- Post-sekondarja
- Gradwat
- Postgradwat

## 28. Indiċi tal-Massa tal-Ġisem

Piż (kg)	Tul (m)	BMI = kg/m <sup>2</sup>

Klassifikazzjoni	Kategorija tal-BMI (kg/m <sup>2</sup> )
Piż baxx	< 18.5
Piż normali	18.5 – 24.9
Piż żejjed	25.0 – 29.9
Obezi Klassi I	30.0 – 34.9
Obezi Klassi II	35.0 – 39.9
Obezi Klassi III	≥ 40

## 29. It-tipjip

### c) X'inhu l-istat tat-tipjip tieghek?

- Tpejjep attwali (mur Q5b)
- Kont tpejjep

Speċifika kemm ilu li waqft tpejjep: \_\_\_\_\_

- Qatt ma pejjipt (mur Q6)

### d) Jekk tpejjep bhalissa, kemm tpejjep sigaretti?

- 1-5 sigaretti kuljum
- 6-10 sigaretti kuljum
- 11-20 sigarett kuljum
- 1-2 pakketti ta' sigaretti kuljum
- Ipejjep soċjalment
- Oħrajn

Speċifika: \_\_\_\_\_

### 30. Konsum ta' Alkohol

**c) Tixrob l-alkohol?**

- Iva (mur għal Q6b)
- Qatt ma **kkunsmajt** alkohol (mur għal Q7)
- Nru

**d) Jekk iva, x"nhu l-konsum medju ta' alkohol fil-gimgha f'unitajiet ta' alkohol (ara Appendix)?**

### 31. Attività Fizika

Matul perjodu ta' **7 ijiem** tipiku, kemm-il darba tagħmel eżerċizzju tat-tipi msemmija, jew simili, għal **iktar minn 15-il minuta** waqt il-ħin liberu tiegħek? Ikteb fuq kull linja n-numru xieraq.

	<b>Hinijiet fil-Gimgha</b>
<b>Eżerċizzju qawwi - Qalb Thabbat malajr</b> Eżempji: Ġirja, jogging, hockey, futbol, squash, basketball, judo, għawm vigoruż, ċikliżmu qawwi fuq distanzi twal	_____
<b>Eżerċizzju Moderat - Mhux Eżawrjenti</b> Eżempji: Mixi mgħagġel, baseball, tennis, ċikliżmu faċli, volleyball, badminton, għawm faċli, żfin	_____
<b>Eżerċizzju Hafif - Sforz Minimu</b> Eżempji: Yoga, qwas, sajd, bowling, golf (mingħajr ma tuża karrettun), mixi faċli	_____

Adapted from: Godin G. The Godin-Shephard Leisure-Time Physical Activity Questionnaire. Health and Fitness Journal of Canada. 2011;4(1):18-22.

### 32. Espożizzjoni ghax-xemx

#### c) Kemm tqatta” hin fix-xemx kuljum?

- Inqas minn 5 minuti
- 5 – 30 minuta
- Aktar minn 30 minuta

#### d) Ammont ta “wiċċ tal-ġilda espost

- Wiċċ u idejn
- Wiċċ, idejn u dirgħajn
- Wiċċ, idejn u riġlejn
- Wiċċ, idejn, dirgħajn u riġlejn

### 33. Fototip tal-ġilda Fitzpatrick

Karatteristiċi tipiċi	Kapaċità tal-ikkunzar	Tip tal-ġilda	
Ġilda ċara ħafna	Dejjem tinħaraq, qatt ma tismar	I	
Ġilda ċara	Dejjem tinħaraq, xi kultant tismar	II	
Ġilda inqas ċara	Kultant tinħaraq, dejjem jismar	III	
Ġilda kannella ċara	Rari tinħaraq, dejjem jismar	IV	
Ġilda kannella skura	Qatt ma tinħaraq, dejjem jismar	V	
Ġilda kannella skura ħafna	Qatt ma tinħaraq, dejjem jismar	VI	

Adapted from Azevedo M, Bandeira L, Luza C, Lemos A, Bandeira F. Vitamin D Deficiency, Skin Phototype, Sun Index, and Metabolic Risk Among Patients with High Rates of Sun Exposure Living in the Tropics. J Clin Aesthet Dermatol. 2018;11(8):15-18

### 34. Użu ta' protezzjoni mix-xemx

#### d) Kemm-il darba tapplika sunblock?

- Kuljum
- Fix-xhur tas-sajf meta twettaq attivitajiet fil-beraħ, darba biss
- Fix-xhur tas-sajf meta twettaq attivitajiet fil-beraħ, f'intervalli regolari kif issuġġerit skond il-fuljett tal-prodott
- Qatt
- Oħrajn

Speċifika: \_\_\_\_\_

#### e) Tapplika protezzjoni mix-xemx fiż-żoni kollha esposti?

- Iva
- Nru

#### f) Jekk le, fejn tapplika l-harsien mix-xemx?

- Wiċċ
- Wiċċ u dirgħajn
- Armi
- Oħrajn

Speċifika: \_\_\_\_\_

### 35. Ko-morbiditajiet

- Mard tal-awtoimmunita

Speċifika: \_\_\_\_\_

- Mard kardjovaskulari

Speċifika: \_\_\_\_\_

- Mard kroniku tal-fwied

- Mard konjittiv

Speċifika: \_\_\_\_\_

- Dijabete



Speċifika t-tip: \_\_\_\_\_

- Mard gastrointestinali

Speċifika: \_\_\_\_\_

- Mard infettiv

Speċifika: \_\_\_\_\_

- Kancer

- Sindromu metaboliku

Speċifika: \_\_\_\_\_

- Osteoporozzi

Speċifika l-istorja ta' waqgħat jew ksur: \_\_\_\_\_

- Mard tal-kliewi

- Disturb tas-Saħħa Mentali

Speċifika: \_\_\_\_\_

- Kundizzjonijiet tal-gilda

Speċifika: \_\_\_\_\_

- Uġiġħ

Speċifika: \_\_\_\_\_

**36. Qatt kellek il-livelli tal-vitamina D tiegħek ittestjati?**

- Iva (mur Q13)

- Le (mur Q15)

**37. Min irreferik għall-ittestjar tal-vitamina D?**

- Tabib

- Spiżjar

- Oħrajn

Speċifika: \_\_\_\_\_

**38. Kif ġie analizzat il-kampjun tad-demem tieghek?**

- Analizi tal-laboratorju
- Point-of-care test

**39. Inti interessat li jkollok il-livelli tal-vitamina D tieghek ittestjati?**

- Iva (mur għal Q16)
- Le (mur għal Q17)

**40. Staġun tal-ġbir tal-kampjuni tad-demem fingerstick**

- Rebbiegħa
- Sajf
- Ħarifa
- Xitwa

**41. Tahseb li jkollok livelli xierqa ta' vitamina D huwa essenzjali għas-saħħa ġenerali?**

- Naqbel ħafna
- Naqbel
- Newtrali
- Ma naqbilx
- Ma naqbilx ħafna

**42. Qatt hadt xi forma ta' suppliment tal-vitamina D?**

- Iva (mur Q19)
- Le (mur Q21)

**43. Min tak parir biex tiehu suppliment tal-vitamina D?**

- Inizjattiva personali
- Ħabib
- Media informattiva
- Spiżjar
- Tabib
- Oħrajn

Speċifika: \_\_\_\_\_

**44. Kont avżat biex tiehu suppliment tal-vitamina D wara test tad-demmm?**

- Iva
- Le

**45. Sa fejn taf, il-livelli attwali ta “vitamina D tieghek huma**

- Defiċjenti (< 10 ng/mL)
- Insuffiċjenti (10 – 30 ng/mL)
- Suffiċjenti (> 30 ng/mL)
- Tossiku (>100 ng/mL)
- Ma nafx

**46. Ikel rikk fil-Vitamina D**

**c) Kemm-il darba tikkonsma ikel rikk fil-vitamina D (eż. bajd, laham ahmar, hut, faqqiegh, ġobon irkotta)?**

- Kuljum
- < 3 darbiet fil-ġimgħa
- Ma nikkunsmax

**d) Tfittex ikel li huwa msahħah bil-Vitamina D?**

- Iva
- Nru

**47. Suppliment ta” Vitamina D**

**d) Bhalissa qed tiehu xi forma ta” suppliment tal-Vitamina D?**

- Iva
- Le (mur għal Q24)

**e) X"doża ta" supplement ta" Vitamina D qed tiehu?**

- Prodotti tal-ħalib imsahħah bil-Vitamina D
- 400IU kuljum
- 1000IU kuljum
- 2000IU kuljum
- 3000IU kuljum
- 4000IU kuljum
- 5000IU kuljum
- 10,000IU fil-gimgħa
- 50,000IU fil-gimgħa
- Ohrajn: \_\_\_\_\_

**f) Għal kemm żmien ilek tiehu d-doża msemmija hawn fuq?**

- < 3 xhur
- 3-6 xhur
- 6-12-il xahar
- 12-24 xahar
- > 24 xahar

**48. Medikazzjoni**

**c) Bhalissa qed tiehu xi mediċini jew supplementi ohra?**

- Iva
- Nru

**d) Jekk iva, jekk joghġbok speċifika**

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# Standard Operating Procedure

*Developed for this Study*

## STANDARD OPERATING PROCEDURE

SOP NUMBER	SOP TITLE
<b>1</b>	<b>ACRO BIOTECH. INC VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)</b>

### PART 1

<b>Author</b>  <b>Catherine Anne Busuttil</b> Doctorate in Pharmacy Student - Pharmacy Department	
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### PART 2

<b>Approver</b>  <b>Dr Francesca Wirth</b> Lecturer - Pharmacy Department	<b>Approver</b>  <b>Prof. Lilian M Azzopardi</b> Head of Department - Pharmacy Department
<b>Approver</b>	

### PART 3

<b>Authoriser</b>  <hr/>	<b>Date of Issue:</b>  <b>Date of next revision:</b>
--------------------------------	--

### PART 4 (To be filled in by OOS, QSU or RSSD)

<input type="checkbox"/> This procedure has been revised and is no longer valid as from:  (Write date)	<input type="checkbox"/> Date of NEXT REVISION is extended until:  (Max. 4 years)	<input type="checkbox"/> SOP rendered obsolete on:  (Write date)
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SOP NUMBER <b>1</b>	SOP TITLE <b>ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)</b>
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SOP NUMBER	SOP TITLE
1	ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)

## 1. Reason for Revision

- 1.1. New SOP.

## 2. Purpose and Scope

- 2.1. This Standard Operating Procedure (SOP) applies to the staff and students using the Acro Biotech Inc. Vitamin D Rapid Test Cassette in the Pharmacy Practice Resource Unit (PPRU) at the Department of Pharmacy, University of Malta.
- 2.2. To describe the procedure for specimen collection and preparation, performing a test, quality control, interpretation of results and maintenance of Acro Biotech Inc. Vitamin D Rapid Test Cassette.

## 3. Definitions

- 3.1. **Buffer:** Used to obtain a valid result by washing the blood up the test strip. The buffer bottle should be held vertically to ensure correct drop size and the number of drops added to the cassette need to be counted.
- 3.2. **Capillary Dropper:** Used to collect a sample of blood from the finger of the subject and to transfer the collected blood to the cassette.
- 3.3. **Colour Card:** Used to read the result by comparing the T line intensity with the card provided in the kit.
- 3.4. **Control Region:** Area within the test cassette where a colour change may be observed confirming that the test has worked properly.
- 3.5. **Fingerstick Whole Blood Specimen:** A procedure in which a finger is pricked with a lancet to obtain a small quantity of capillary blood for testing purposes.
- 3.6. **Lancet:** Used for the collection of capillary blood from the fingertip in adult subjects.
- 3.7. **Test Region:** Area within the test cassette where a colour change giving a diagnostic result may be observed.
- 3.8. **Vitamin D Rapid Test Cassette:** A rapid chromatographic immunoassay for the semi-quantitative detection of 25-hydroxyvitamin D (25 (OH) D) in human fingerstick whole blood at a cut-off concentration of  $30 \pm 4\text{ng/mL}$ . This assay provides a preliminary diagnostic test result and can be used to screening for Vitamin D deficiency.

## 4. Responsibilities

- 4.1. The members of the Department of Pharmacy (staff and students) are responsible for following this SOP.
- 4.2. The designated Laboratory Officer or Laboratory Assistant is responsible for ensuring that this SOP is followed.

SOP NUMBER	SOP TITLE
1	ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)

## 5. Health and Safety Requirements

Refer to SOP PHR-004-01 Health and Safety: Point-of-care Testing for general health and safety practices, and SOP PHR-005-01 ACCU-CHEK Safe-T-Pro Plus for the correct disposal of blood contaminated lancets.

- 5.1. Use the test kit for professional teaching and research practices or *in vitro* diagnostic practices.
- 5.2. Do not use the Vitamin D Rapid Test kit beyond its expiration date. When used for demonstration purposes, the results from the test are to be discarded if the test kit has been used beyond its expiration date.
- 5.3. Store the Vitamin D Rapid Test kit within the sealed pouch up to time of use.
- 5.4. Consider all specimens to be potentially hazardous material and therefore handled as one would infectious agents.

## 6. Procedure

(Refer to Diagram 1 and 2)

### 6.1. Specimen Collection and Preparation

- 6.1.1. Wash the subject's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
- 6.1.2. Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
- 6.1.3. Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
- 6.1.4. Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- 6.1.5. Add the fingerstick whole blood specimen to the test by using a capillary dropper.
- 6.1.6. Touch the end of the capillary dropper to the blood, do not squeeze the bulb of the dropper, the blood migrates into the dropper through the capillarity to the black line indicated on the dropper. Avoid air bubbles.
- 6.1.7. Squeeze the bulb to dispense the whole blood to the specimen area of the test cassette.
- 6.1.8. Perform the test immediately after the fingerstick whole blood has been collected.

### 6.2. Performing a Test

- 6.2.1 Allow the test specimen, buffer and/or controls to reach room temperature (15°C – 30°C) prior to testing.
- 6.2.2 Remove the test cassette from the sealed pouch and use it as soon as possible.



SOP NUMBER <b>1</b>	SOP TITLE <b>ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)</b>
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- 6.2.3 Place the test cassette on a clean and level surface.
- 6.2.4 To use a capillary dropper, fill the capillary tube and transfer approximately 20µL of fingerstick whole blood specimen to the specimen area of test cassette, then add 2 drops of buffer (approximately 80µL) and start the timer.
- 6.2.5 Wait for the coloured line(s) to appear. Read results at 10 minutes by comparing the T line intensity with provided colour card. *Note: It is suggested not to use the buffer beyond 30 days after opening the vial unless for demonstration purposes.*
- 6.2.6 Interpret the result according to Table 1 and 2 to determine the concentration of Vitamin D in the subject's body.

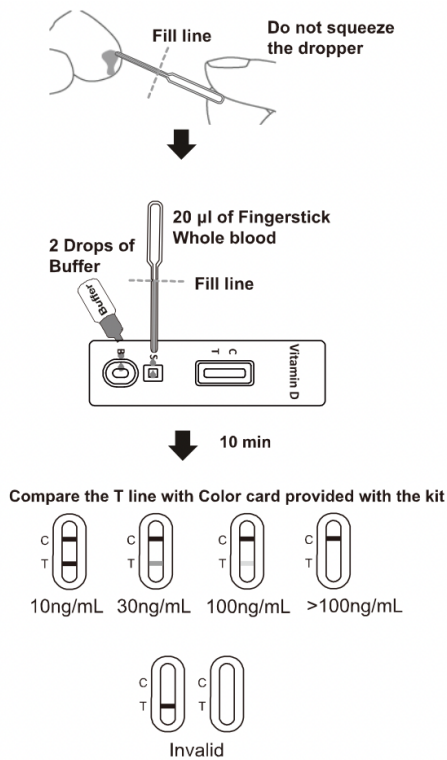


Diagram 1: Illustrative Diagram of Procedure

SOP NUMBER <b>1</b>	SOP TITLE <b>ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)</b>
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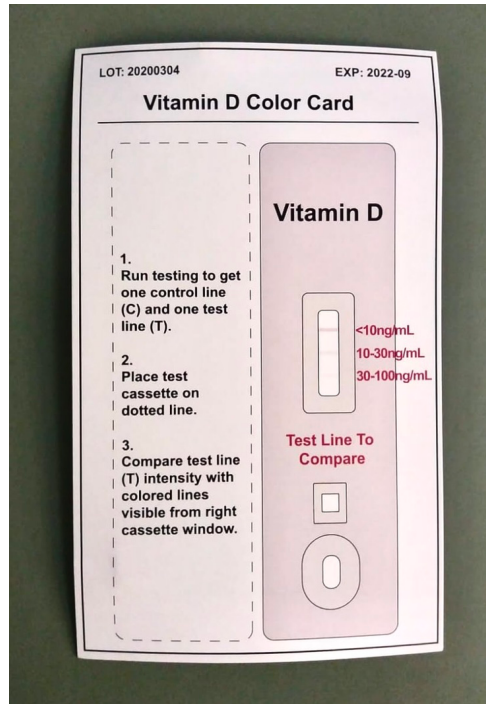


Diagram 2: Colour Card

SOP NUMBER <b>1</b>	SOP TITLE <b>ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)</b>
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### 6.3. Interpretation of Results

Refer to Diagram 1 and compare the T line intensity with ‘Vitamin D Colour Card’ provided with the kit.

Result	Interpretation
<b>Deficient</b>	Two distinct coloured lines appear. One is in the control region (C) and another should be in the test region (T). The line intensity in the test region (T) is equal to or darker than 10ng/mL line depicted on colour card provided with the kit.
<b>Insufficient</b>	Two coloured lines appear. One is in the control region (C) and another should be in the test region (T). The line intensity in the test region (T) is darker than the 30 ng/mL line depicted on the colour card provided with the kit and lighter than 10 ng/mL line depicted on Colour card provided with the kit.
<b>Sufficient</b>	Two coloured lines appear, one line should be always in the control region (C) and faint coloured line appears in the test region (T). The line intensity in region (T) is darker than the 100 ng/mL line depicted on the Colour card and lighter than 30 ng/mL line depicted on colour card.
<b>Toxicity Levels</b>	Only one coloured line appears in the test region (C), no coloured line appears in the test region (T). Note: Always compare the T line intensity with “Vitamin D Colour card” and interpret results accordingly.
<b>Invalid</b>	Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Table 1: Interpretation of Results

	Package Insert	
	<i>Serum 25OHD</i>	
	<i>ng/mL</i>	<i>nmol/mL</i>
<b>Vitamin D Deficiency</b>	0-10	0-25
<b>Insufficiency</b>	10-30	25-75
<b>Sufficiency</b>	30-100	75-250
<b>Toxicity</b>	>100	>250

Table 2: Categories of low vitamin D (25-hydroxyvitamin D) levels – Adapted from Smith et al., 2017

Smith LM, Gallagher JC. Dietary Vitamin D Intake for the Elderly Population: Update on the Recommended Dietary Allowance for Vitamin D. *Endocrinol Metab Clin North Am.* 2017;46(4):871-884.

SOP NUMBER	SOP TITLE
1	ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)

#### 6.4. Quality Control

An internal procedural control is included in the test. A coloured line appearing in the control line region (C) is an internal positive procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique. Control standards are not supplied with this kit; however, it is recommended that standard controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

#### 6.5. Maintenance

6.5.1. Store as packaged in the sealed pouch at room temperature or refrigerated (2-30°C) until use.

### 7. References

- 7.1. Eriksen EF, Glerup H. Vitamin D deficiency and aging: implications for general health and osteoporosis. *Biogerontology*. 2002;3(1-2):73-7.
- 7.2. Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. *Altern Med Rev*. 2005;10(2):94-111.
- 7.3. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc*. 2006; 81(3):353-73.
- 7.4. Moyad MA. Vitamin D: a rapid review. *Dermatol Nurs*. 2009;21(1):25-30, 55.

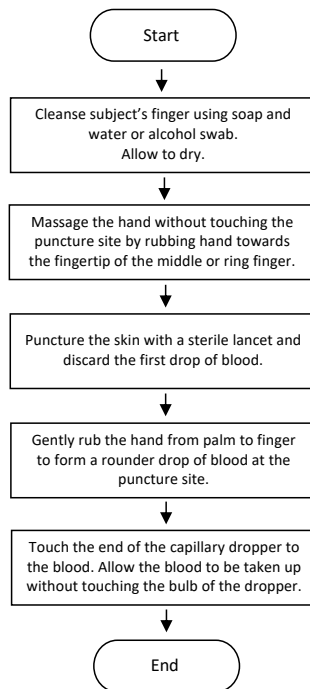
### 8. List of Appendices/Worksheets

- 8.1. Appendix 1: Flow Chart – Specimen Collection and Preparation
- 8.2. Appendix 2: Flow Chart – Performing a Test
- 8.3. Appendix 3: Limitations, Expected Values and Performance Characteristics

SOP NUMBER <b>1</b>	SOP TITLE <b>ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)</b>
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**APPENDIX 1**

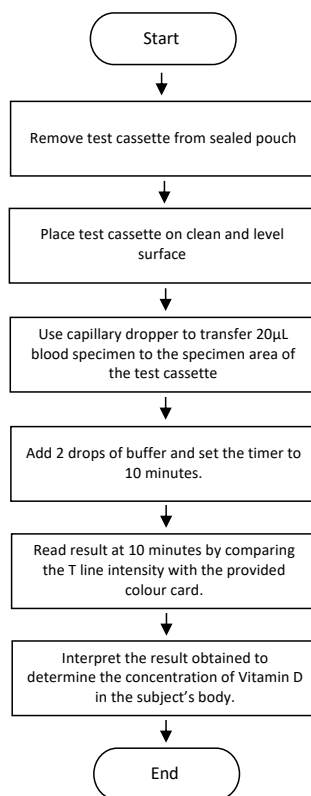
**FLOW CHART – SPECIMEN COLLECTION AND PREPARATION**



SOP NUMBER <b>1</b>	SOP TITLE <b>ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)</b>
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**APPENDIX 1 (Continued)**

**FLOW CHART – PERFORMING A TEST**



SOP NUMBER	SOP TITLE
<b>1</b>	<b>ACRO BIOTECH INC. VITAMIN D RAPID TEST CASSETTE (WHOLE BLOOD)</b>

### APPENDIX 3

#### LIMITATIONS

- The Vitamin D Rapid Test Cassette provides only a semi-quantitative analytical result. A secondary analytical method must be used to obtain a confirmed result.
- It is possible that technical or procedural errors, as well as other interfering substances in the whole blood specimen may cause erroneous results.
- The Cut-off for the test is 30 ng/mL with a deviation range of  $\pm 4$  ng/mL.
- As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
- Other clinically available tests are required if questionable results are obtained.

#### EXPECTED VALUES

The Vitamin D Rapid Test Cassette (Whole Blood) has been compared with predicate Device (Vitamin D Rapid Test), demonstrating an overall accuracy of 93.8%.

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**PERFORMANCE CHARACTERISTICS**

Accuracy

The Vitamin D Rapid Test Cassette has been compared with predicate device (Vitamin D Rapid Test). The following results were tabulated:

Method	Predicate Device (Vitamin D Rapid Test)				
	Results	<i>Deficient</i>	<i>Insufficient</i>	<i>Sufficient</i>	Total Result
<b>Vitamin D Rapid Test Cassette</b>	<i>Deficient</i>	4	4	0	8
	<i>Insufficient</i>	0	64	2	66
	<i>Sufficient</i>	0	0	23	23
	Total Result	4	68	25	97
	<b>Accuracy</b>	<b>&gt;99.9%</b>	<b>94.1%</b>	<b>92.0%</b>	<b>93.8%</b>

Intra-Assay

Within-run precision has been determined by using 3 replicated of four specimens: 10ng/mL, 30ng/mL, 45ng/mL and 100ng/mL specimens. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 3 independent assays on the same 4 specimens: 10ng/mL vitamin D, 30ng/mL vitamin D, 45ng/mL vitamin D and 100ng/mL vitamin D standard samples. Three different lots of the Vitamin D Rapid Test Cassette have been tested using these specimens. The specimens were correctly identified >99% of the time.

Sensitivity and Cross-Reactivity

The Vitamin D Rapid Test Cassette can detect levels of Vitamin D in human fingerstick whole blood as low as 30ng/mL. The addition of Vitamin A, B, C, E, K and M showed no cross-reactivity.



## Action Plan

### 1) Initiate lifestyle advice to all participants

Disseminate “Vitamin D Information Leaflet” (Appendix A)

### 2) Vitamin D level – **Deficient**

Refer patient to physician, using Referral Note (Appendix B)

- a. Consider whether the patient is about to initiate medication related to bone disorders. Provide the below comments in Referral Note:

In such cases, consider loading regimen of approximately 300,000 IU of colecalciferol orally over 6-10 weeks. Suggest follow up assessment of serum calcium and Vitamin D testing 4 weeks after completing loading regimen for Vitamin D

Suggest reassessment of serum Vitamin D 3 months following completion of loading regimen if patient is still symptomatic (Cowan et al., 2017; Theobald et al., 2021).

- b. Consider calcium intake:

- i. If calcium intake is sufficient, based on assessment of oral supplementation being taken by the patient, providing an intake of  $\geq 700$ mg daily, include in comments suggestion to initiate colecalciferol 800 – 2000 IU orally daily (Cowan et al, 2017; Theobald et al., 2021).

- ii. If calcium intake is insufficient, include in comments suggestion to consider calcium and Vitamin D orally. For patients with a **deficient** Vitamin D level, a supplementary dose of 1000IU of

Vitamin D is recommended to be considered (Theobald et al., 2021).

- iii. Include in comments that Vitamin D levels should be repeated after 3-6 months on recommended replacement therapy (Cowan et al. 2017).

### 3) Vitamin D level – **Insufficient or Sufficient**

Initiation of Referral Note (Appendix B) to physician is recommended **only** if one or more of the following applies:

- i. Fragility fracture, osteoporosis or high fracture risk is present
- ii. Patient is being treated with medication for bone disease
- iii. Patient is symptomatic for Vitamin D deficiency (Appendix C)
- iv. Increased risk of developing Vitamin D deficiency, examples:
  - Inadequate exposure to UVB light
  - Inadequate dietary intake of foods rich in Vitamin D
  - Metabolic factors including age, BMI, chronic hepatic disease and/or chronic renal impairment
  - Gastrointestinal diseases such as Crohn’s disease, inflammatory bowel disease, coeliac disease, etc.
  - Individuals with darker skin types
  - Pregnant or lactating women
  - Use of “anticonvulsants, rifampicin, cholestyramine, anti-retrovirals, glucocorticoids” (NICE 2014; Cowan et al., 2017; Rockwell et al., 2018; Essig et al., 2020; Theobald et al., 2021).

A Result Sheet (Appendix D) will be given to patients when no Referral Note is required.

## References

Cowan A, Pugh R, McCaughey A. Treatment of Vitamin D Deficiency in Adults v2 [Internet]. Wirral Clinical Commissioning Group: National Health System; 2017 [updated 2020 June; cited 2021 Mar 1. Available from: [https://mm.wirral.nhs.uk/document\\_uploads/guidelines/Vitamin%20D%20Guidelines%20for%20Adults%20v2.pdf](https://mm.wirral.nhs.uk/document_uploads/guidelines/Vitamin%20D%20Guidelines%20for%20Adults%20v2.pdf)

Essig S, Merlo C, Reich O, Trottmann M. Potentially inappropriate testing for Vitamin D deficiency: a cross-sectional study in Switzerland. *BMC Health Serv Res.* 2020;20(1):1097. Published 2020 Nov 27. doi:10.1186/s12913-020-05956-2

National Institute of Health and Care Excellence. Covid-19 Rapid Guideline: Vitamin D (NG187). Public Health England: Scientific Advisory Commission on Nutrition; 2020 [cited 2021 June 11]. Available from: <https://www.nice.org.uk/guidance/ng187/resources/covid19-rapid-guideline-vitamin-d-pdf-66142026720709>

National Institute of Health and Care Excellence (NICE). Vitamin D: Supplement use in specific population groups (PH56). Public Health England: Scientific Advisory Commission on Nutrition; 2014 [updated 2020 August 2017; cited 2021 June 11]. Available from: <https://www.nice.org.uk/guidance/ph56>

Theobald J, Sherwood N, Catt L, Sutton J, Nottingham Osteoporosis Group. Vitamin D Management in Adults V5.0 [Internet]. Nottinghamshire Area Prescribing Committee: National Health System; 2021 [cited 2021 June 11]. Available from: <https://www.nottsapc.nhs.uk/media/1248/vitamin-d-guidelines-adults.pdf>

Rockwell M, Kraak V, Hulver M, Epling J. Clinical Management of Low Vitamin D: A Scoping Review of Physicians' Practices. *Nutrients.* 2018;10(4):493.

# Appendix A

## Vitamin D Information Leaflet in English

### Vitamin D

Information Leaflet

#### How does Vitamin D contribute to health?

- Maintenance of healthy **bones, muscles, and teeth**
- Facilitates **uptake of calcium**
- Boosts **immunity**

Signs of low vitamin D levels include aches and pains and fatigue, but most individuals may not experience any symptoms.

#### How may Vitamin D levels be increased naturally?



The action of sunlight on our skin is the main source of Vitamin D.

Spending around **15 minutes in sunlight, 3 times a week**, having the face and forearms exposed to the sun, may help to boost Vitamin D levels.

Avoid being exposed to sunlight during hours where there is a very high UV index to avoid sunburn and other consequences of excessive sun exposure.



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Vitamin D may be obtained through consumption of **foods which are rich** in Vitamin D.

These foods include **egg yolk, red meat, oily fish, mushrooms, ricotta cheese** and **foods fortified** with vitamin D such as some dairy products, juices, or breakfast cereals.



#### Which dose of Vitamin D is suggested for healthy adults and children?

Supplementation of Vitamin D should be initiated under the supervision and recommendation of a doctor or pharmacist who are able to suggest the right dose of vitamin D for you!



#### Vitamin D supplementation is suggested to:

- Persons with **limited sun exposure**
- Individuals who wear clothes to **cover up most of their skin** outdoors
- Persons with **darker skin** types
- **Pregnant or lactating women**
- **Immunocompromised** patients with **risk factors** for developing vitamin D deficiency

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Catherine Anne Busuttill  
B.Sc Pharm Sci (Hons.) M.Pharm  
Doctorate in Pharmacy Dissertation

September 2021

## Vitamin D Information Leaflet in Maltese

### Vitamina D

Fuljett ta' Informazzjoni

#### Il-Vitamina D kif tikkontribwixxi ghas-sahha?

- Manutenzjoni tas-sahha tal-**ghadam, muskoli u snien**
- Tiffacilita l-**assorbiment tal-kalcju**
- Isahha l-**immunita**

Sinjali ta' livelli baxxi ta' vitamina D jinkludu ugigh u gheja, izda hafna individwi jistghu ma jesperjenzaw l-ebda sintomi.

#### Kif jistghu jizdiedu l-livelli ta' Vitamina D b'mod naturali?



L-azzjoni tad-dawl tax-xemx fuq il-gilda taghna hija s-sors ewlieni tal-Vitamina D.

Li tqatta madwar **15-il minuta fid-dawl tax-xemx, 3 darbiet fil-gimgħa**, bil-wicc u d-dirghajn esposti ghax-xemx, jista jghin biex izid il-livelli ta' Vitamina D.

Evita li tkun espost ghax-xemx matul is-sghat fejn l-indici tal-UV ikunu gholjin hafna biex tevita hruq u konsegwenzi ohrain ta' esponiment ghax-xemx ecCESSIV.



Il-Vitamina D tista' tinkiseb permezz ta' konsum ta' **ikel li hu rikkk** fil-Vitamina D.

Dan l-ikel jinkludi **l-isfar tal-bajd, laham ahmar u fwied, hut zejtni, faqqiegh, irkotta, ikel fortifikat bil-Vitamina D** (eż. xi prodotti tal-halib, meraq tal-frott jew cereali).



#### Liema doza ta' Vitamina D hija ssuggerita ghal adulti u tfal b'sahhithom?

Supplimentazzjoni ta' Vitamina D ghandha tinbeda taht is-supervizjoni u r-rakkomandazzjoni ta' tabib jew spizjar li kapaçi jissuggerixxu l-ahjar doza ta' vitamina D ghalik!



#### Is-supplimentazzjoni tal-Vitamina D hija ssuggerita lil:

- Persuni b'espozizzjoni limitata ghax-xemx
- Individwi li jilbsu hwejjeg biex jghattu hafna mill-gilda taghhom
- Persuni b'tipi ta' gilda skura
- Nisa tqal u nisa li qed iredgħu
- Pazjenti immunokompromessi b'fatturi ta' riskju għall-izvilupp ta' deficjenza ta' vitamina D.

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Doctorate in Pharmacy Dissertation

Settembru 2021

## Appendix B

### Referral Note

**Point of Care Test:** Vitamin D Rapid Test Cassette (Acro Biotech, Inc.) – Semi-Quantitative

**Name:** \_\_\_\_\_ **ID number:** \_\_\_\_\_

**Date:** \_\_\_\_\_ **Lot No:** \_\_\_\_\_

#### Test Result

- Deficient
- Insufficient
- Sufficient
- Toxicity

#### Vitamin D Level

Serum 25OHD	ng/ml
Deficient	0-10
Insufficient	10-30
Sufficient	30-100
Toxicity	>100

**Reason for Referral:**

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**Comments:**

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September 2021

## Appendix C

### Clinical features of Vitamin D Deficiency and Osteomalacia

- “Gradual onset and persistent bone pain without preceding mechanical injury (frequently in back, ribs or lower limbs)
- Fragility fracture
- Proximal muscle weakness (difficulty with stairs, getting up off the floor or standing after sitting in a low chair, waddling gait) or muscle pain
- Carpopedal spasm, tetany, seizures or irritability due to hypocalcaemia and requiring urgent treatment
- Osteopenia on plain radiograph
- Low bone density on dual energy x ray absorptiometry scan (does not equate to osteoporosis)”

Adopted from: Theobald J, Sherwood N, Catt L, Sutton J, Nottingham Osteoporosis Group. Vitamin D Management in Adults V5.0 [Internet]. Nottinghamshire Area Prescribing Committee: National Health System; 2021 [cited 2021 June 11]. Available from: <https://www.nottsapc.nhs.uk/media/1248/vitamin-d-guidelines-adults.pdf>

# Appendix D

## Result Sheet

### Result Sheet

**Point of Care Test:** Vitamin D Rapid Test Cassette (Acro Biotech, Inc.)

**Date:** \_\_\_\_\_

**Lot No:** \_\_\_\_\_

#### Test Result

- Deficient
- Insufficient
- Sufficient
- Toxicity

#### Vitamin D Level

Serum 25OHD	ng/ml
Deficient	0-10
Insufficient	10-30
Sufficient	30-100
Toxicity	>100

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Doctor of Pharmacy Dissertation – Vitamin D Point-of-Care Testing

January 2022



## **Appendix 4: Dissemination of Results**

Abstract submitted for 2022 ACCP Global Conference on Clinical Pharmacy, San Francisco, USA

### **Abstract**

Community Pharmacist-Led Vitamin D Point-of-Care Testing

Catherine Busuttil, Francesca Wirth, Lilian M. Azzopardi

Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta, Malta

#### **Service or Program:**

The aim was to establish a framework for community pharmacist-led Vitamin D point-of-care testing (POCT). Vitamin D POCT devices were appraised. A framework consisting of a Data Collection Sheet, Standard Operating Procedure for POCT and Action Plan for patient management and collaborative practice was developed and validated amongst an interprofessional expert panel. The feasibility of implementation of the developed framework was tested within a community pharmacy setting on 80 participants recruited by convenience sampling.

#### **Justification/Documentation:**

With increasing awareness of the relevance of Vitamin D to immunomodulation, patient and general practitioner requests for access to Vitamin D level testing increased. A need was identified for the provision of a service that ensures patient safety, quality and reliability of the

testing process. The review identified a semi-quantitative POCT to assess Vitamin D (sensitivity 4ng/ml, cost €6 per kit), which conforms with EU medical device regulations and is feasible to be applied within the community pharmacy setting. The POCT results were validated against the laboratory-driven test (gold standard) for 20 patients ( $\kappa = 0.84$ ,  $p < 0.001$ ). Feasibility testing of the Vitamin D POCT framework was carried out on 80 participants in a community pharmacy; 49 participants had insufficient and 8 participants had deficient Vitamin D levels.

**Adaptability:**

The development of the Vitamin D POCT framework enables standardisation of pharmacist-led service provision of Vitamin D POCT testing and is feasible to be implemented as a service provision in the community pharmacy setting.

**Significance:**

The developed framework has led to the implementation of an innovative service of POCT of Vitamin D levels with appraisal of patient identification of risks and recommended personalised action plan. The community pharmacist-led service expands clinical pharmacy provision in the primary care setting and responds to a health service need that was identified with respect to Vitamin D level testing.

# Poster



L-Università ta' Malta  
Faculty of  
Medicine & Surgery

Department  
of Pharmacy

## COMMUNITY PHARMACIST-LED VITAMIN D POINT-OF-CARE TESTING

Catherine Anne Busuttill, Francesca Wirth,  
Lilian M Azzopardi  
catherine.busuttill.13@um.edu.mt

### SERVICE OR PROGRAM

To establish a framework for community pharmacist-led Vitamin D point-of-care testing (POCT).

#### Process

1. Appraisal of Vitamin D POCT devices
2. Validation of selected Vitamin D POCT by comparing results with gold standard (Table 1)
3. Development of Vitamin D POCT framework including **risk assessment** and **action plan for patient management**
4. Feasibility testing of developed framework in a community pharmacy setting on 80 participants recruited by convenience sampling (Figures 1-3)

### SIGNIFICANCE

The community pharmacist-led service developed responds to an identified health service need with respect to Vitamin D POCT. This pharmacist-led approach to Vitamin D POCT aims to:

- **Reduce economic burden** on healthcare facilities
- **Add value to clinical pharmacy provision in primary care**
- **Benefit patients** through harmonisation of Vitamin D analysis, coupled with identification of risks and a personalised action plan (Figure 4).

Table 1: POCT vs. Gold standard (N=20)

Vitamin D Test Result	POCT	Gold Standard
Deficient	1	2
Insufficient	17	16
Sufficient	2	2

Cohen's kappa (K) = 0.84

Figure 2: Vitamin D Levels Tested Previously (N=80)

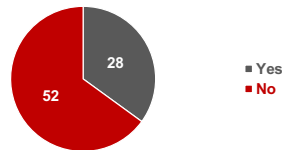


Figure 1: Vitamin D POCT Results (N=80)

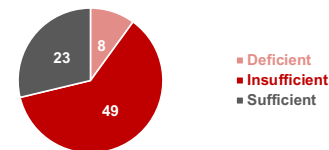


Figure 3: Presence of Metabolic Disorder vs. Vitamin D Level (N=80)

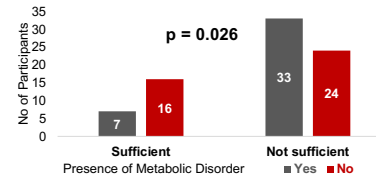
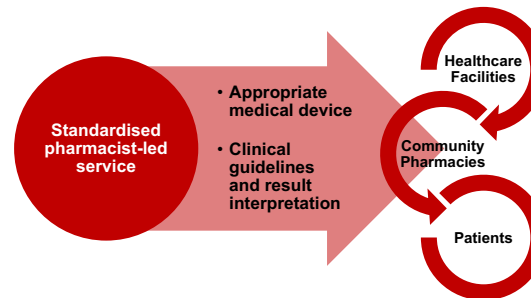


Figure 4: Significance of framework



### JUSTIFICATION

- With increased awareness on the relevance of Vitamin D to immunomodulation, patient and general practitioner requests for access to Vitamin D testing increased. A need was identified for service provision in primary care that **ensures patient safety, quality and reliability** in the testing process.
- The service developed identified a semi-quantitative POCT to assess Vitamin D (sensitivity 4ng/ml, cost US\$6 per kit) which conforms with EU Medical Device Regulations and is feasible to be applied within community pharmacy.
- The POCT results were **validated** against the laboratory-driven test (gold standard) for 20 patients. Concordance was observed between the two methods (k=0.84) (Table 1).
- Figure 1 presents the Vitamin D POCT results undertaken in community pharmacy, with 57 participants showing deficient or insufficient Vitamin D levels (Figure 1). Statistical significance was observed between presence of metabolic disorders and deficient or insufficient Vitamin D level (p=0.026) (Figure 3).

### ADAPTABILITY

Development of the **Vitamin D POCT framework** enables **standardisation of pharmacist-led** Vitamin D POCT testing and is **feasible** to be implemented as a **service** in community pharmacy.

Financial Support: University of Malta Research Grant (PHRP03\_20), Brown's Pharma Ltd.