CLINICAL STUDY PLAN

Version 6, 08 December 2016

Clinical Study to Evaluate the Safety and Performance of the Calcivis® System for Identifying Active Demineralization on Tooth Surfaces

CAL-02-2014

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Revision History

VERSION / DATE	REVISION CHANGES
Version 1 / 11 November 2015	Original Version
Version 2, 04 January 2016	**Section 15.3 – Informed Consent** The sentence regarding "The patients's General Practitioner will be informed of their participation in the study" has been removed further to Ethics review
Version 3, 08 February 2016	**Section 1 – Synopsis – Patient Population** **Section 1 - Inclusion and Exclusion Criteria** **Section 7.4 – Inclusion Criteria and Reference Table** Inclusion criteria no. 2 changed to include inscisors
Version 4, 03 May 2016	**All pages - Footers** - changed on every page to reflect amended version and date **Page 1 – Front Page** – version number and date changed **Page 3 - Investigator Signature Page -** reference to version and date changed in Investigator declaration **Page 9 – 1. Synopsis – Study Device - Intended Use and Indications** and **Page 18 – 5.2 General Description -** changes to description of Calcivis Disclosing Solution Kit to reflect syringe needles replaced by needleless adaptors **Page 24 – 5.9 Device Labelling and Storage -** changes to description of contents of Calcivis Disclosing Solution Kit and Calcivis Application Kits and change of storage condtions for the Calcivis Disclosing Solution Kit from 2 to 8^0C to Room Temperature **Page 29 – 8.2 Study Visit 1 – Preparation Procedures for Teeth** and **Page 48 - Appendix 2 – Tooth Cleaning Protocol** – amended from cleaning teeth with prophylaxix paste to cleaning teeth with water or dental paste
Version 5, 19 October 2016	**All pages - Footers** - changed on every page to reflect amended version and date **Page 1 – Front Page** – version number and date changed **Page 3 - Investigator Signature Page -** reference to version and date changed in Investigator declaration

	Page 9 – 1. Synopsis – Study Device - Intended Use and Indications and **Page 19 – 5.2 General Description** addition of description of Calcivis Disclosing Solution (Photoprotein) multi-use format**,** and clarification to description of Calcivis Disclosing Solution single-use format and Calcivis Application Kit. **Page 25 – 5.9 Device Labelling and Storage –** addition of description of contents of Calcivis Multi-use Disclosing Solution (photoprotein) Kit and corresponding storage condtions. **Page 26 – 6.1 Risk Analysis -** addition of possible safety concerns and mitigations of multi-use format of Photoprotein **Page 30 – 8.2 Study Visit 1** – **Preparation of the Calcivis System -** clarification of storage conditions for the Calcivis multi-use Disclosing Solution (Photoprotein)
Version 6, 08 December 2016	**All pages - Footers** - changed on every page to reflect amended version and date **Page 1 – Front Page** – version number and date changed **Page 4 - Investigator Signature Page -** reference to version and date changed in Investigator declaration **Page 13 – Study Investigators –** removal of Site 02 (Charles Ormond) and addition of Site 05 (Agnieszka Nohawica) **Page 31** – **8.5 Independent Investigator Review** – Table amended to take into account removal of Site 02 and addition of Site 05

INVESTIGATOR SIGNATURE PAGE

Clinical Study to Evaluate the Safety and Performance of the Calcivis System for Identifying Active Demineralization on Tooth Surfaces
CAL-02-2014

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Designated Sponsor Representative: Adam Christie, BSc, MBA
CEO, Calcivis Ltd

Print Name: ADAM CHRISTIE

Signature: _____ Date: 8 DEC 2016

Chief Investigator / Principal Investigator: Neil Shanks, BDS, MJDF
Downie, Harper & Shanks Dental Practice
55 Captain's Road
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I have read this Clinical Study Plan (CAL-02-2014, v6, 08 December 2016) and understand its requirements. I agree to conduct the study as described herein and will not deviate from the Clinical Study Plan without prior written approval of the Sponsor or designee. Any Clinical Study Plan changes, other than administrative, must be made by written amendment to the Clinical Study Plan and will not be implemented until approved by the Research Ethics Committee.

Print Name: NEIL SHANKS

Signature: _____ Date: 8.12.16

INVESTIGATOR SIGNATURE PAGE

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Principal Investigator: Elaine Downie, BDS,
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Print Name: ELAINE DOWNIE

Signature: _____ Date: 8th Dec 2016

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I have read this Clinical Study Plan (CAL-02-2014, v6, 08 December 2016) and understand its requirements. I agree to conduct the study as described herein and will not deviate from the Clinica Study Plan without prior written approval of the Sponsor or designee. Any Clinical Study Plan chang other than administrative, must be made by written amendment to the Clinical Study Plan and will be implemented until approved by the Research Ethics Committee.

Print Name: F MORRISON

Signature: _____ Date: 13.12.16

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I have read this Clinical Study Plan (CAL-02-2014, v6, 08 December 2016) and understand its requirements. I agree to conduct the study as described herein and will not deviate from the Clinical Study Plan without prior written approval of the Sponsor or designee. Any Clinical Study Plan changes, other than administrative, must be made by written amendment to the Clinical Study Plan and will not be implemented until approved by the Research Ethics Committee.

Print Name: STEPHEN MARTIN

Signature: _____ Date: 20/12/16

INVESTIGATOR SIGNATURE PAGE

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I have read this Clinical Study Plan (CAL-02-2014, v6, 08 December 2016) and understand its requirements. I agree to conduct the study as described herein and will not deviate from the Clinical Study Plan without prior written approval of the Sponsor or designee. Any Clinical Study Plan changes, other than administrative, must be made by written amendment to the Clinical Study Plan and will not be implemented until approved by the Research Ethics Committee.

Print Name: AGNIESZKA NOHAWICA

Signature: _____ Date: 08/12/2016

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TABLE OF CONTENTS

1. SYNOPSIS

Study Name and Unique Number	**Clinical Study to Evaluate the Safety and Performance of the Calcivis System for Identifying Active Demineralization on Tooth Surfaces – CAL-02-2014**
Study Objectives	**Primary Objectives** Performance of the Calcivis System, as measured by the presence or absence of elevated luminescence on the surface of the tooth determined from intra-oral image mapping of that surface (with or without a visible lesion). Safety of the Calcivis System, as measured by the collection of all adverse events The **Secondary Objective** of the study is: To assess the usefulness of the Calcivis System images, as a communication tool between patient and dentist, as measured by Questionnaires and / or Patient Visual Analogue Scales.

Study Device - Intended Use and Indications	The Calcivis Caries Activity Imaging System comprises: ***Calcivis Imaging Kit - Administration and Imaging device*** Consists of: ▪ Calcivis Intra-oral Imaging Device ▪ Device cradle ▪ Calcivis (Imaging) Software on DVD/CD ▪ Calcivis Instruction Manual ***Accessory - Calcivis Disclosing Solution Kit – single use*** Consists of: ▪ Calcivis Disclosing Solution (Freeze dried in vials) ▪ Water for reconstitution ▪ Syringe ▪ Needleless adaptors **and / or** ***Accessory – Calcivis Disclosing Solution (Photprotein) Kit - Multi-use*** ***Consists of:*** • Calcivis Photoprotein (Disclosing Solution) - Freeze dried in a vial • Vial of Calcivis Diluent (Water for reconstitution) • Single-use Device Syringes (sterile prior to opening) • Vial Adaptors x 2 ***Accessory – Calcivis Application Kit*** ***Consists of:*** • Single-use Calcivis Applicators x10 The Calcivis Caries Activity Imaging System is intended to be used by dental healthcare professionals on patients (6 years and older) with, or at risk of developing, caries lesions on coronal tooth surfaces. The Calcivis Caries Activity Imaging System is indicated for use to provide images of active demineralization on tooth surfaces, as an aid to the assessment and diagnosis of caries lesions.
Study Design	Prospective, multi-site, non-randomised, post-approval clinical study

Patient Population:	Eligible patients will be recruited from routine, general dental practices who have one unrestored, accessible, free smooth buccal surface on a canine or incisor , away from the gingival surface, identified with no visible lesion (coded ICDAS 0), and / or one unrestored, accessible, erupting or erupted molar or pre-molar with a visible lesion identified (coded ICDAS 2 or 3) in a plaque stagnation area.
Inclusion and Exclusion Criteria	<u>Inclusion criteria</u> 1. Patient must be ≥ 6 years old 2. Patient must have one unrestored, accessible, free smooth buccal surface on a canine or incisor, away from the gingival surface identified with no visible lesion (coded ICDAS 0) – ref. table on page 27 and / or 3. Patient must have one unrestored, accessible, erupting or erupted molar or pre-molar with a visible lesion identified (coded ICDAS 2 or 3) in a plaque stagnation area – ref. table on page 27 4. Patient and / or parent or guardian must be willing and able to give written informed consent 5. Patient and / or parent or guardian must be willing and able to adhere to study schedule <u>Exclusion criteria</u> 1. Any Patient with recent tooth bleaching (within previous two weeks of imaging with the Calcivis System) 2. Any Patient having on-going re-mineralization treatment including, but not limited to high concentration prescription fluoride toothpaste 3. Any patient with a fixed orthodontic appliance 4. Any patient currently taking part in a clinical research study, or has taken part in a clinical research study in the previous three months 5. Pregnant and / or nursing mothers

Statistical Rationale	The study will assess the agreement between the Calcivis System and dentist rating of suitable teeth into two categories: 'no visible lesions' or 'active lesions'. Based on previous study data and expert opinion, 'No visible lesions' are expected to correspond to 'no luminescence' in at least 70% of cases. Similarly, 'active lesions' are expected to correspond to 'luminescence' in at least 70% of cases. For the purpose of sample size calculations, the percentage agreement for each of the two categories are jointly considered as measures of agreement. That is, both measures will need to show statistically significant agreement for the study to be considered successful. The study is sized to provide at least 90% power to reject the null hypothesis of chance agreement (50%). Statistical hypothesis tests will be 1-sided and conducted at the 2.5% significance level. This requires a mimimum of 81 evaluable teeth of each category.
Number of Patients / Investigator	A minimum of 17 and a maximum of 18 **teeth** from each tooth population are required from each Investigator (a total of 85 to 90 teeth from each tooth population). Therefore per Investigator - if all patients have one of each tooth population, a minimum of 17 patients per Investigator will be recruited and if all patients only have one of each tooth population, a maximum of 36 patients will be recruited. Therefore the number of patiens recruited to the study will range from 85 to 180.
Number of Sites	Four (4)

Sites and Investigators	**Site 01:** Neil Shanks (Chief Investigator) Downie, Harper & Shanks Dental Practice 55 Captains Road Edinburgh, EH17 8HP Scotland Tel: 0131 664 2184 Elaine Downie (Investigator) Downie, Harper & Shanks Dental Practice 55 Captains Road Edinburgh, EH17 8HP Scotland Tel: 0131 664 2184 **Site 03:** Fraser Morrison (Investigator) Bathgate Smile Centre Whitburn Road Bathgate, EH48 2SS West Lothian Scotland Tel: 01506 656563 **Site 04:** Steve Martin (Investigator) Marchmont Periodontics 18 Thirlestane Road Edinburgh, EH9 1AN Scotland Tel: 0131 447 4159 **Site 05:** Agnieszka Nohawia (Investigator) Bosco Dental Studio 3/25 Thorny Crook Terrace Dalkeith, EH22 2RF Scotland Tel: 0131 654 9316
Duration of Procedure	Preparation for Imaging is expected to take approximately one minute per tooth with imaging itself taking less than one second. All eligible teeth per patient will be imaged at the same dental visit
Follow-Up	7 to 14 days post imaging

Duration of Study	The overall study period is expected to take 10 months - 3 months for Ethics Committee and R & D approval, 2 to 3 months for recruitment and study procedures, and 4 months for final follow up, data collection and analysis.
Regulatory Status	The Calcivis System is a Class IIa Medical Device which is CE marked in the EU.

2. SCHEDULE OF EVENTS

	Screening	Visit 1			Visit 2	Indendent Review
	Pre-Imaging	Pre-Imaging	Imaging	Post-Imaging	Post-Imaging (7 to 14 days)	
Identification of patients / issue of Patient Information Sheet	X					
Written Informed Consent		X				
Demographics		X				
Inclusion / Exclusion Criteria		X				
Oral Hygiene information		X				
Tooth ID (ICDAS score / activity status) and cleaning		X				
Intra-oral colour photographs – tooth surface only		X				
Calcivis System - Images / disclosing solution / images			X			
Adverse Events (intra-oral colour photograph if relevant)		X	X	X	X	
Image interpretation – Dentist			X			
Image review – Dentist and Patient			X			
Patient Questionnaire / Visual Analogue Scale			X			
User Questionnaire			X			
Second Investigator / Dentist Image Review						X

3. INTRODUCTION

3.1 Background

Dental caries (tooth decay) is a significant clinical and public health problem. Caries is associated with localised demineralization of the tooth surface which may lead to progressive loss of tooth structure and associated pain and morbidity (Selwitz et al, 2007[1]). The development of caries lesions involves a net mineral loss of dental tissue, (mediated by the acid in dental bacterial plaque on the surface of the tooth), in what is initially principally a sub-surface phenomenon. Detecting, assessing, diagnosing and treating such hard tissue lesions is a core activity in dentistry. (Pitts NB, 2009[2]; Fejerskov O, et al 2008[3]; Paris S, 2013[4]). The main detection and diagnostic aids for caries have long been visual inspection and the use of a probe, together with X-ray images.

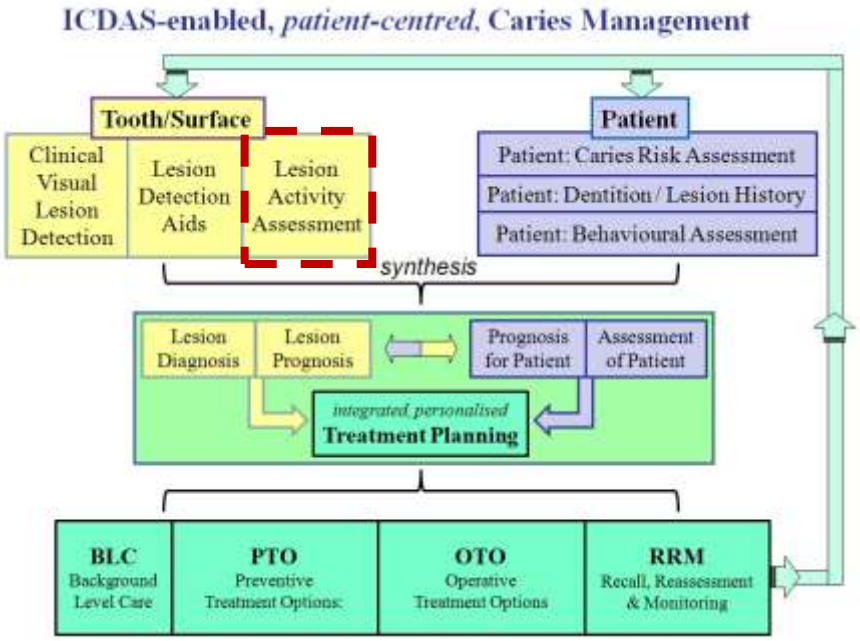
While a number of technologies have been developed to aid caries lesion detection, this step represents only part of the problem for clinicians in relation to clinical decision-making. Determination of the activity status of a lesion is required in order to assess the treatment needs for this lesion. Current methods of clinical assessment of lesion activity are problematic and involve the clinician's subjective assessment and/or the monitoring of lesion behaviour over time using a specific detection technique such as radiography and/or a combination of factors involved in caries aetiology to produce an algorithm weighted in an attempt to discriminate between active and inactive lesions. The assessment of the activity status of a specific lesion in a single patient visit remains difficult (Ekstrand K et al, 2009[5]; Ekstrand K, Martignon S, 2013[6]) since it currently involves the visual assessment of the subtle differences in the physical characteristics of the lesion surface. Not all dental caries lesions progress to cavitation. The determination of which early 'white spot' lesions will progress can currently only be achieved with any significant degree of accuracy by monitoring over time. Such observing of a lesion risks its progression beyond the stage where preventive interventions are appropriate to a point at which more complex treatments are involved. There are significant differences in the treatment requirements for active and inactive caries lesions, with consequent marked differences in costs and outcomes for the patient, dentist and third-party payer. There is thus a need to develop a technique to aid dentists in identifying the activity status of caries lesions at a single visit in order to optimise the use of non-invasive preventive therapies, as compared to operative / surgical interventions, which tend to be more expensive and can have long-term negative clinical consequences (Qvist V 2008[7]; Longbottom C et al, 2009[8]; Meyer-Lueckel H et al, 2013[9])

3.2 Overview of Technology

The technology underlying the Calcivis System was developed to help address the unmet need in relation to the determination of caries lesion activity status. The Calcivis system combines a sensitive custom intraoral imaging device and a bioluminescent marker which produces light in the presence of free calcium ions as they are released from actively demineralizing areas of a tooth surface. The images produced by the system are effectively maps of demineralization activity across that surface.

This information is potentially valuable in the context of a systematic approach to caries detection and assessment such as ICDAS (International Caries Detection and Assessment System) – Appendix 2. This study will therefore be set within the ICDAS framework. (Pitts NB and Ekstrand KR, 2013[10]; www.icdas.org).

Figure 1. Overview of ICDAS Caries Management Process



The ICDAS concept is that the use of a standardised system, based on best available evidence for detecting early and later stage caries severity, should lead to the acquisition of better quality information which could then be used to inform decisions about appropriate diagnosis, prognosis, and clinical management of dental caries at both the individual and public health levels.

The Calcivis System provides information that informs Lesion Activity Assessment (in yellow with Red border), in Figure 1 above. An active lesion is a lesion that is actively deteriorating (demineralization is outstripping remineralization). Currently there are no marketed products that allow direct assessment of active demineralization at a single patient visit.

The device is intended to be used on visually accessible occlusal and free smooth surfaces and to provide the dental professional with additional information to the clinical visual (and other clinical, radiographic and additional technology-derived) data in order to help enable the clinician to assess / determine the caries activity status of a tooth site / surface. This assessment will aid the clinician in determining the management option for each caries lesion. Fundamentally, a clinician has three possible options when confronted with a caries lesion – he can: 1) monitor it, 2) apply preventive treatments such as sealants and remineralization therapies 3) drill and fill it. The combination of the assessment of the extent of the lesion, in terms of its depth towards the dental pulp, and its activity status will be critical in determining the clinician's treatment decision from these 3 options.

3.3 Risk / Benefits Rationale

The potential benefits from using the Calcivis System relate to the clinician being able to make a more informed decision about lesion activity status – the more accurate the information relating to lesion activity status, the more likely an appropriate treatment decision will be made. The potential risks from using the device relate to the device providing a false positive signal with the consequent increased potential for the clinician deciding the lesion requires either non-operative preventive

therapy or a restoration / filling, i.e. drilling, (the latter being unlikely since, if there is no cavitation present, the current guidelines indicate a restoration is not required).

Previous laboratory research on recently extracted teeth has demonstrated that there is strong correlation between positive light signals generated by the early Calcivis technology and caries lesion activity status, as assessed by a clinician, as well as the physical characteristics of the surface of active lesions.

The results of the previous clinical study on the advanced prototype of the Calcivis System confirmed the device to be safe in clinical use, and provided an acceptable level of "correlation" between the Investigators rating of sound and unsound teeth using ICDAS coding and the Calcivis System. In particular the results showed a higher level of correlation for the sound teeth, (83.9%) meaning the chances of false positives are low. It may be that some of the teeth considered sound had sub-clinical but actively demineralizing lesions.

The feedback from both user and patient questionnaires, provided useful information on some of the design features, which have now been incorporated in to the commercial device which will be easier to use.

3.4 Study Rationale

Therefore the purpose of this clinical study is to evaluate both the clinical safety and performance of the next generation Calcivis System for identifying active demineralisation on the surfaces of teeth on a patient population ≥ 6 years old.

4. STUDY OBJECTIVES AND ENDPOINTS

4.1 Primary Objectives

Performance of the Calcivis System, as measured by the presence or absence of elevated luminescence on the surface of the tooth determined from intra-oral image mapping of that surfaces of teeth (with or without a visible lesion).

Primary Safety Objective

Safety of the Calcivis System, as measured by the collection of all adverse events

4.2 Secondary Objective

To assess the usefulness of the Calcivis System images, as a communication tool between patient and dentist, as measured by Questionnaires and / or Patient Visual Analogue Scales.

5. STUDY DEVICE

5.1 Intended Use

The Calcivis Caries Activity Imaging System is intended to be used on patients by dental healthcare professionals on patients (6 years and older) with, or at risk of developing, caries lesions on coronal tooth surfaces.

The Calcivis Caries Activity Imaging System is indicated for use to provide images of active demineralization on tooth surfaces, as an aid to the assessment and diagnosis of caries lesions.

5.2 General Description

The Calcivis Caries Activity Imaging System comprises:

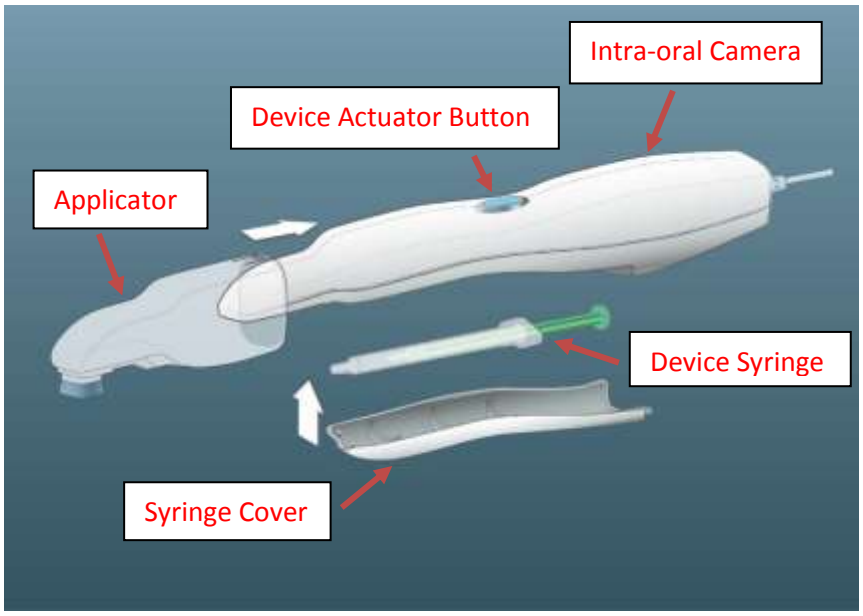
Calcivis Imaging Kit - Administration and Imaging device
Consists of:

- Calcivis Intra-oral Imaging Device
- Device cradle
- Calcivis (Imaging) Software on DVD/CD
- Calcivis Instruction Manual

The software main functions are; to initiate imaging (visible and luminescent), to save and retrieve digital images for display, and the overlaying of black and white images with luminescence images to map location of luminescence, representing elevated calcium levels, to the tooth surface.

The software requirements are detailed in the Instructions For Use.

Figure 2: Calcivis Intra-oral Imaging Device



Accessory - Calcivis Disclosing Solution Kit – single-use
Consists of:

- Calcivis Disclosing Solution (Freeze-dried in vials)
- Water for reconstitution
- Syringe
- Needleless adaptors

and / or

Accessory – Calcivis Disclosing Solution (Photprotein) Kit - multi-use
Consists of:

- Calcivis Photoprotein (Disclosing Solution) - Freeze dried in a vial
- Vial of Calcivis Diluent (Water for reconstitution)
- Single-use Device Syringes (sterile prior to opening)
- Vial Adaptors x 2

Accessory – Calcivis Application Kit
Consists of:

- Single-use Calcivis Applicators x10

5.3 Device Use

The device can be used for screening purposes but in this study the device will be used as a site specific technique intended to be used where a dentist has already detected a potential carious lesion. The dentist will have carried out a standard oral examination to detect and assess potential caries lesions. Where a potential lesion is detected and the dentist would like more information about that lesion, i.e. is there on-going active loss of calcium ions (de-mineralization) from that lesion, use of the Calcivis System may be appropriate. In normal operation, the dentist will image a tooth before application of the Disclosing Solution using the Calcivis intraoral camera. The Disclosing Solution will then be applied to the same tooth and another image will be taken with the Calcivis camera. The image is processed by the software then displayed on a PC monitor and can then be printed and / or stored to hard disk or incorporated on to dental practice imaging management systems. The system is fully automated so that both images are taken within less than 0.5 seconds (including the automated application of the disclosing solution).

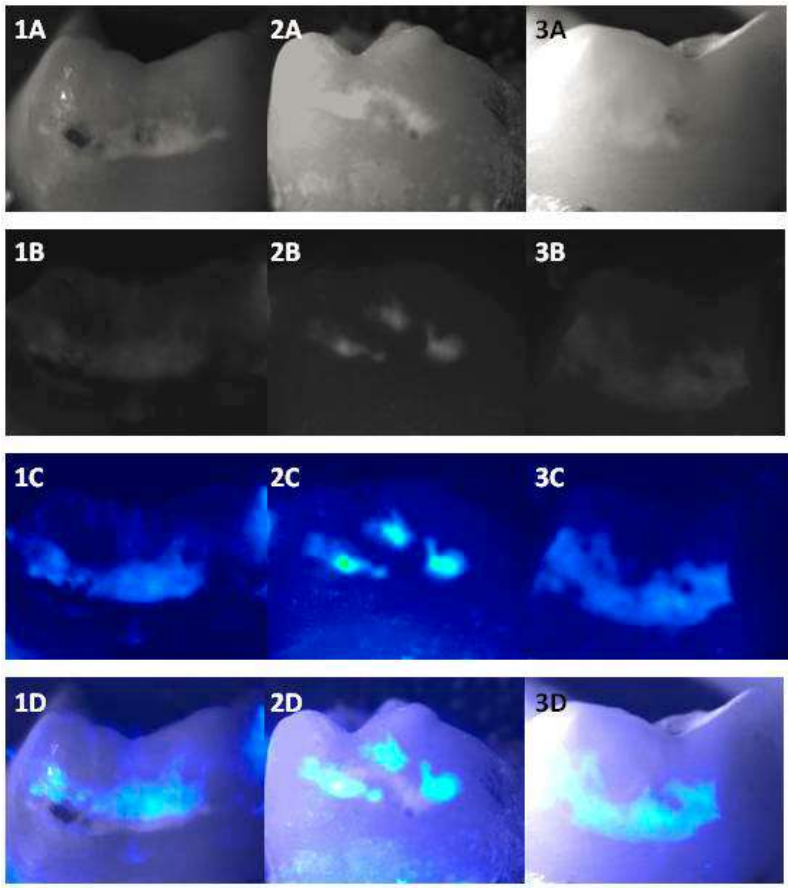
Full details for the operation of the Calcivis System is supplied in the Instructions For Use.

5.4 Device Technology

The Calcivis System technology involves imaging localised calcium loss from demineralizing tooth surfaces, as evidenced by the luminescence, or light emission (low level in visible spectrum), produced from the ionic calcium interacting with the Disclosing Solution (containing photoprotein), after it is applied to the tooth surface. The light emission results from a chemical reaction between the photoprotein and free Calcium, in contrast to fluorescence based technologies which require an excitatory light source.

The Disclosing Solution is placed on to a tooth and where ionic calcium is present light is generated which the intraoral camera detects and records. The majority of the light signal is captured in less than 0.1 second. Software is used to overlay the visible and luminescent images in order to highlight regions where calcium ions are present, providing an easy to use interface, thereby providing de-mineralization maps of the tooth surfaces which are interpreted by the Dentist. Figure 3 below summarizes this process.

Figure 3: Summary of visualization of free calcium by Calcivis System



*Typical white spot lesions can be seen on the visible images of freshly extracted teeth (**1A to3A** – black and white images).*

*Luminescent images of the same three teeth (**1B to 3B**) and false-coloured luminescent images (**1C to 3C**). Both these types of images* show the observed pattern of luminescence from the tooth surface after the addition of disclosing solution

*Merged images of the black and white images with the false-coloured luminescent images (**1D to 3D**) which show* the positioning of the luminescent pattern on the tooth surface for each of the three teeth.

5.5 Device Manufacture

The Calcivis system intra-oral imaging device will not normally come into contact with the skin, teeth or mucosa of the patient. It will be covered by a single use, disposable applicator, which may come into contact with the skin, teeth and mucosa of the patient, to protect the patient from any possible cross-contamination from the multi-use camera unit. The imaging unit will be cleaned thoroughly between use with hospital grade disinfectant wipes. The camera connects directly with the USB of a PC and incorporates a CMOS system, Light Emitting Diode (LED) – visible light and a lens to capture the image. Images can be transferred to the PC for display by the software.

The Disclosing Solution comprises a lyophilized, powdered recombinant bioluminescent protein. By supplying the disclosing solution lyophilized, it maximizes the shelf life and specificity of the bioluminescent capability. The powder will be supplied non-sterile but low bioburden and will be reconstituted immediately before use with deionized water (also supplied). A total of 2.5 µg (100µg/ml in 25 µl application) of photo-protein is dispensed per application in the disclosing solution.

5.6 Regulatory Status

EU

The **Calcivis System** is classified in the EU as a Class IIa Medical Device according to the Medical Device Directive.

The Calcivis System comprises three components as follows:

Device (Intra-oral Camera) - administration and Imaging device

Class IIA Rule 11 for administration of disclosing solution,
Class I Active Rule 12 for imaging
Class I Active Rule 12 for Software
(Therefore the highest class is Class IIA Rule 11)

Disclosing Solution – lyophilised protein and water for reconstitution

Class I Rule 5 invasive (via natural orifice) and transient. Intended to bind with free calcium ions at the tooth surface and emitted light (in visible spectrum) captured by the imaging system.

Disposables – applicator, syringe and needle

Class I Rule 5 invasive (via natural orifice). Syringe and needle to be provided sterile until open.

USA

The Calcivis System falls under 'diagnosis, prevention, monitoring, treatment or alleviation of disease, and does not achieve its action by pharmacological, immunological or metabolic means'. The Calcivis technology involves the detection and display of a chemical reaction as part of caries lesion activity assessment, which includes imaging localised calcium loss from demineralizing surfaces. This is only one of several factors which can be used to diagnose caries. Elevated levels of calcium ions can also be a result of a number of different causes (dental erosion, dental fluorosis and enamel hypomineralisation). The dentist therefore has to consider all potential causes before making a diagnosis. Thus, the Calcivis System has the potential for showing activity of caries and is an aid to the dentist, and not diagnostic *per se*.

5.7 Device Safety

Clinical and Pre – Clinical Data

As the mode of action of the Calcivis Device is detection of free calcium on the tooth surface it is relatively simple to check this function using calcium solutions of varying concentration in wells and confirm using extracted teeth. Release testing of the disclosing solution includes a functional assay employing stopped-flow technology. The stopped flow process uses fixed syringes to rapidly (within a few nanoseconds) dispense set volumes of known concentration of the luminescent marker and 1mM calcium chloride to a mixing chamber, from which light output is collected and assessed. The intra-oral imaging device can be set up in a dark box or "mock mouth" with either wells of calcium solution or extracted teeth to confirm detection of calcium and expected demineralization maps on carious teeth.

A Clinical Evaluation was performed, taking into account the "mock-mouth" bench-top studies, pre-clinical studies on extracted teeth and clinical studies of similar comparator devices. In addition, pre-clinical toxicology studies on the Disclosing Solution (cytotoxicity, oral toxicity, oral irritation and sensitisation tests) were also carried out according to ISO10993 and American Dental Association guidelines. The Clinical Evaluation Report concluded that sufficient clinical data was available to support the safety and performance of the Calcivis System, without the need for a specific clinical study.

The Calcivis System was CE marked in December 2013, following submission of the Technical File.

In May 2014, the first formal Post-Approval Clinical Study was performed in adults (16 to 25 yrs old) using the advanced prototype Calcivis System. The Primary Objectives of the study were to assess the Performance and Safety of the Calcivis System, and the Secondary Objective was to assess user experience with the Calcivis System (both User and Patient). A total of 42 patients were recruited to the study from three UK, General Dental Practices, to obtain a total of 31 evaluable patients. Eligible patients were those with interpretable images of both a tooth with no visible lesion identified (ICDAS Code 0) and a tooth with a suspected visible lesion identified (ICDAS Code 1, 2 or 3). Images from 11 patients were not eligible for interpretation due to confounding factors including ambient light ingress, gross saliva contamination and poor disclosing solution coverage on the tooth surface. All these issues have been addressed in the design phase for the new commercial Calcivis System which is the subject of this study.

All 42 patients were included in the Safety Population – two adverse events were recorded – bleeding gums and slight gum abrasion – both were device-related, non-serious and asymptomatic to the patients with no action required. One other adverse event was recorded as a device deficiency when the device jammed and did not dispense fluid.

31 patients were included in the Agreement Population – analysis of the level of agreement between the ICDAS scores and elevated luminscence, (Y/N) as defined by the Investigators.

For the primary study objectives, the study results concluded that: -

1. The Calcivis System was safe in clinical use as determined by the low number and minor nature of the adverse events recorded.

2. Analysis of the overall level of agreement between elevated luminescence and the presence of lesions predicated as active by the Investigators showed agreement in 47 of the 65 teeth imaged (72.3 %) which was statistically significant, not due to chance and above the expected level of 70%.

   (Of the 31 teeth identified by the Investigators with no visible lesions, 26 showed no elevated luminescence using the Calcivis System – corresponding to a negative percentage agreement of 83.9%. Of the 34 teeth with visible lesions identified by the Investigators, 21 showed elevated luminescence using the Calcivis System – corresponding to a positive percentage agreement of 61.8%).

A recent paper by Alves et al ([11]) indicates an association between the stage of eruption and activity status of caries. As summarized in table below:

Stage of Eruption	1	2	3	4
sound	526	108	814	694
inactive non-cavitated	9	5	148	164
active non-cavitated	184	43	179	48
Total caries non-cavitated	193	48	327	212
% act non- cavitated	95	90	55	23

stage 1, partially erupted occlusal surface;

stage 2, fully erupted occlusal surface, <1/2 crown exposed; and

stage 3, fully erupted occlusal surface, >1/2 crown exposed

stage 4. Full occlusion

The teeth examined in the clinical study were predominantly stage 3 i.e. *fully erupted occlusal surface, with greater than half the crown exposed* with expected activity in approx. 55% of lesions.

For the secondary study objective, the study results concluded that: -

Although one patient recorded 'marked pain' on the Patient Questionnaire, the overall feedback was positive with 91.4% of patients recording their experience with the Calcivis System as 'good' or 'very good'. 88.1% of patients reported that seeing the images of their teeth and having the dentist explain their situation was 'helpful' or 'very helpful'. 65% of the dentists recorded their overall user experience with the Calcivis® System to be 'good'.

5.8 Device Accountability

All study devices will be accounted for. Master Device Accountability records will be maintained by the Sponsor for all device components – intra-oral imaging device kit, disclosing solution kits and application kits.

Each site will be provided with one intra-oral imaging device for use in the study, and a sufficient number of disclosing solution kits and application kits, for the target number of patients to be recruited. Additional supplies of all three components will be available, should they be required.

Each site will maintain Device Delivery and Return documentation. All intra-oral imaging devices, and unused disclosing solution kits and application kits will be returned to Calcivis Ltd at the end of the study period.

Returned study devices / components will be checked against the Master Device Accountability records by the Sponsor. Any discrepancies will be documented and investigated.

5.9 Device Labelling and Storage

The Intra-Oral Imaging Device Kit will be supplied as a single unit, non-sterile and will be identified by a unique serial number.

The single-use Disclosing Solution Kits will be supplied non-sterile in boxes, each box containing 10 vials of the freeze-dried protein, water for reconstitution, individually wrapped, sterile, off- the- shelf syringes, and individually wrapped, sterile, off- the- shelf needless adaptors. Each vial will be identified by a unique lot number.

The Calcivis multi-use format will be supplied in a non-sterile in box, containing 1 x 5ml vial of freeze-dried photoprotein and individualy wrapped adaptor, 1 x 5ml vial of diluent and individually wrapped adaptor, 10 x individulally wrapped sterile, off-the-shelf single-use syringes and 10 x individually wrapped single-use applicators.

The Application Kits will be supplied in boxes of ten, each kit containing an individually wrapped applicator. The individual Applicators will be identified by a unique lot number.

The Intra-Oral Imaging Device and the Application Kits will be stored at room temperature. The Disclosing Solution Kits, containing the lyophilised protein and de-ionised water for reconstitution, will be stored in a secure area not above 250 C For the Calcivis multi-use format, once reconstituted the vial of Photoprotein will be stored at 2 to 80 C, up to a maximum of 4 weeks.

5.10 Study / Device Training

Full training on all aspects of the study will be provided by the Sponsor as follows:

- Identification of teeth according to ICDAS caries scoring system and caries lesion activity assessment
- Tooth cleaning protocol
- Imaging with standard Intra-oral camera
- Full preparation and use of the Calcivis Sytem according to the manufacturer's Instructions For Use, to include –
    - preparation of the disclosing solution and syringe loading and device activation
    - laptop set-up and image interpretation and storage
- Study procedures and data recording
- Adverse event collection, recording and reporting
- Informed Consent Process

All training will be fully documented for Investigators / Dentists and Dental Nurses. In addition technical support will be available on the study visit days, if required.

6. RISKS AND BENEFITS

6.1 Risk Analysis

Extensive risk analyses have highlighted the following potential areas of safety concern (high impact risks):

1. Integrity of the single use Applicators
2. Deterioration of the Disclosing Solution throughout its shelf life
3. Toxicity of the Disclosing Solution
4. Contamination of vial from re-use (multi-use format only)

These are all considered very low likelihood and have all been adequately addressed by:

1. Integrity testing of applicators in addition to breach requiring a triple fault condition in that previous patient would need to be carrying disease, main device would have to not be cleaned between uses and any contaminant on device would need to penetrate to subsequent patients.
2. Extensive stability studies on the Disclosing Solution
3. ISO 10993 compliant pre-clinical toxicological study and recent clinical study on Disclosing Solution shows no sign of toxicity
4. The multi-use reconstituted solution contains an anti-microbial preservative. In addition , wiping top of Photoprotein vial with alcohol wipes between uses and drawing up of phototprotein with single-use syringes.

6.2 Potential Risks

The Calcivis system has been designed and tested in compliance with ISO 13485 and will only be used by fully trained dental professionals. Any risks associated with the use of the Calcivis System have been analysed as above, and provision made to either reduce or eliminate these risks. Potential risks to the patient include:

- cross-contamination between patients, if not used according to manufacturer's instructions
- hypersensitivity of patient to protein or other components in Disclosing Solution
- over / under application of Disclosing Solution leading to necessity for repeat procedure
- discomfort and / or soft tissue trauma due to presence of intra-oral imaging device in mouth

6.3 Potential Benefits

This study is intended to show the safety and performance of the Calcivis System for evaluating active demineralization on tooth surfaces in a clinical setting. When used to characterise de-mineralization in teeth, it is anticipated that the Calcivis System will benefit both the user (dentist) and patient. Potential benefits to the patient are:

- treatment of early lesions with re-mineralization therapies or sealants to prevent progression to cavitation
- enhanced clinical decision making
- reducing the need for X-rays
- improved communication between dentist and patients and improved potential for prevention of caries

It is therefore anticipated that the overall benefits will outweigh any risks associated with the use of the Calcivis System.

7. STUDY DESIGN

7.1 Overview

This is a prospective, multi-site, non-randomised, post-approval clinical study to assess the use of the Calcivis System for assessing active demineralization on tooth surfaces of teeth in patients 6 years and older. This post-approval clinical study will be conducted under the controlled conditions of this clinical study plan, on eligible patients, in a general dental practice setting.

From the Expert Paper on Caries Activity Assessment, Longbottom C, 2015 ([12]) it concludes that the prevalence of active lesions found on the occlusal surfaces of erupting teeth is affected by the erupting stage status of the teeth – with the prevalence of active lesions greater in the earlier eruption stages.

As a result of these and similar findings the choice of teeth for identifying visible lesions which are potentially 'active' has been limited to erupting and erupted molars and premolars (in which case occlusal surface must be such that, apart from minimal presence of an operculum over the distal marginal ridge, it is clear of the gingivae (gum)).

7.2 Patient Selection and Confidentiality

To ensure poolability of the data from each Investigator, up to 36 patients may be recruited to the study (to obtain a minimum of 17 evaluable images of each tooth population) by five Investigators from four general dental practices. Patients will be identified and selected according to the Inclusion / Exclusion criteria listed below. All patients and / or parent or guardian must provide written informed consent and be willing to adhere to the study schedule, before being entered in to the study.

Patients will be encouraged to complete both Study Visits, however, patients are free to withdraw Consent at any time, irrespective of their initial consent.

Patients may also be withdrawn from the study by the Investigator on the grounds of safety. Any patients who withdraw or are withdrawn from the study will be replaced.

All patients recruited to the study will be identified by a unique study number, comprising the site number and a patient number, to allow any data collected on them to be anonymized. The investigator will maintain a confidential patient identification list of all patients enrolled in the study (by name and patient number). The list will be maintained at the site and will not be retrieved by the Sponsor. There is no randomization required in this clinical study.

7.3 Study Duration

The overall study period is expected to take 10 months - 3 months for Ethics Committee and NHS, R & D approval, 2 to 3 months for recruitment and study procedures, and 4 months for final follow up, data collection and analysis.

7.4 Inclusion and Exclusion criteria

Inclusion criteria

1. Patient must be ≥ 6 years old
2. Patient must have one unrestored, accessible, free smooth buccal surface on a canine or incisor, away from the gingival surface identified with no visible lesion (coded ICDAS 0) – ref. table below

   and / or
3. Patient must have one unrestored, accessible, erupting or erupted molar or pre-molar with a visible lesion identified (coded ICDAS 2 or 3) in a plaque stagnation area – ref. table below
4. Patient and / or parent or guardian must be willing and able to give written informed consent
5. Patient and / or parent or guardian must be willing and able to adhere to study schedule

Tooth status population	Exemplar tooth type and location	Additional criteria
Sound tooth	Canine or incisor Away from gingival margin	Enamel should be shiny and feels hard and smooth when the tip of a probe is moved gently across the surface
Active Lesion	Erupting or erupted Molars Fissure pits of occlusal surface	Surface of enamel should be whitish/yellowish opaque with loss of luster; feels rough when the tip of a probe is moved gently across the surface. Lesion should be in a plaque stagnation area i.e. pits and fissures; approximally, near the gingival surface below the contact point.
Inactive Lesion	Molars and pre-molars Smooth surface away from gingival margin	Surface of enamel should be whitish, brownish or black. Enamel should be shiny and feels hard and smooth when the tip of a probe is moved gently across the surface. For smooth surfaces, the caries lesion will be typically located at some distance from the gingival margin.

Exclusion criteria

1. Any Patient with recent tooth bleaching (within previous two weeks of imaging with the Calcivis System)
2. Any Patient having on-going re-mineralization treatment including, but not limited to high concentration prescription fluoride toothpaste
3. Any patient with a fixed orthodontic appliance
4. Any patient currently taking part in a clinical research study, or has taken part in a clinical research study in the previous three months
5. Pregnant and / or nursing mothers

8. STUDY PROCEDURES

8.1 Screening Procedures

Patients attending routine dental appointments who are identified by the Investigator as meeting all the inclusion / exclusion criteria, will be approached to discuss their possible participation in the study. Initial approach will be by the Dental Nurse to ask the patient and / or parent or guardian, if they would be interested in participating in the study. The study will be explained to them and each patient and / or parent or guardian will be given a copy / copies of the relevant Patient Information Sheet and Consent Forms to read and discuss with others if required. The patient and / or parent or guardian will be asked to confirm their interest in participating in the study by contacting the Dental Practice (no less than 24 hours after being given the Patient Information and Consent Form). They will be given the opportunity to discuss their participation in the study with the Investigator and ask any questions. If the patient and / or parent or guardian is still willing for the patient to participate in the study, they will be asked to return to the dental practice for Study Visit 1, when written Informed Consent will be taken by the Investigator. Patient availability for Study Visit 2 (7 to 14 days post-imaging) will also be checked.

The Site will document all patients and / or parent or guardian provided with the Patient Information Sheet and the outcome, (whether or not recruited to the study) on a Patient Screening / Recruitment Log.

8.2 Study Visit 1

At this visit, written Informed Consent (ref. 15.3) will be taken by the Investigator and the following information collected and procedures carried out.

<u>Pre-Imaging Information collected</u>

Demographics – DoB, ethnicity, relevant medical history and medications

Oral hygiene information - brushing regime, toothpaste and any other dental products used

<u>Preparation of the Calcivis System</u>

The Calcivis System must be prepared according to the manufacturer's Instructions For Use. Single-use Disclosing Solution (Photoprotein) should not be reconstitued more than 2 hours before the first tooth is imaged. Once reconstituted, the multi-use vial of Disclosing Solution (Photoprotein) should be stored at 2 to 80 C, up to a maximum of 4 weeks.

<u>Preparation Procedures for Teeth</u>

Relevant teeth will be identified and recorded for assessment using the Calcivis System as per Inclusion / Exclusion criteria.

All teeth surfaces to be imaged will be cleaned by the Dentist, by brushing with water or dental paste and rinsing with water and an air-water spray from a conventional dental 3-in-1 device, according to the Tooth Cleaning Protocol (Appendix 2). Patients will be asked to rinse out thoroughly with tap water.

Immediately following thorough air-drying of each tooth surface to be imaged, the Investigator will take a colour photograph with a standard intra-oral camera and record the ICDAS score /activity status.

Preparation Procedures for imaging with the Calcivis device

Care must be taken to ensure the surface of the tooth and surrounding area are free from saliva before imaging by the use of appropriate moisture control aids.

Each tooth will be air-dried for 5 to 10 seconds immediately before application of the disclosing solution and Calcivis System imaging.

Imaging with the Calcivis System

For teeth from each category (no lesions, and active lesions) a maximum of one tooth per patient can be imaged.

If a patient has more than one tooth of a particular category (sound tooth [no visible lesion], or tooth with active lesion) the investigator will use best clinical judgement to choose a tooth that most clearly fits the criteria of that particular population tooth type, for example if there is the choice of 2 teeth displaying lesions considered active, each identical except one of the teeth has a lesion in a plaque stagnation area (e.g. pits and fissures) and one more distant from plaque stagnation area, the tooth with the lesion in a plaque stagnation area will be chosen.

If the first image of a selected tooth is not clear for interpretation, it is acceptable to take a second image of that tooth, however no more than two images of any one tooth can be taken.

Recruitment will be stopped for each Investigator when a minimumof 17 evaluable images (as determined by the Investigators) from each tooth population have been obtained.

After all imaging has been completed, patients will be asked to rinse out with tap water.

Any adverse events observed or volunteered by the patient will be recorded. If appropriate a colour photograph will be taken of the adverse event with a standard intra-oral camera.

The images generated with the Calcivis System will be stored digitally on the laptop provided. The software will allow overlay of the two sets of images (before and after application of the disclosing solution) and interpretation of the resulting demineralization map of each imaged tooth will be carried out by the Investigator at the end of imaging.

At the end of the imaging procedures, the Investigator will share the images of the teeth with the patient.

The Calcivis System will be dismantled and cleaned according to the Instructions For Use. All consumables will be disposed according to the Instructions For Use.

Representatives of the Sponsor, Calcivis Ltd, will be available throughout Visit 1 to provide technical support for the use of the Calcivis System, as and when required. Consent will be sought from the patient and / or parent or guardian.

Post-Imaging Questionnaires / Visual Analogue Scales

At the end of the imaging procedures, patients will be asked to remain at the dental practice to complete a Patient Questionnaire / Visual Analogue Scale. Visit 1 is then complete.

In addition, at the end of each imaging study visit, the Investigator and Dental Nurse will each complete relevant sections of a User-Questionnaire

8.3 Study Visit 2 – 7 to 14 days post-imaging

At this visit, a final oral examination will be performed by the Investigator and any adverse events observed or volunteered by the patient will be recorded. If appropriate a colour photograph will be taken of the adverse event with a standard intra-oral camera.

8.4 Future Dental Care

Depending on the results, the Investigator may provide caries preventive advice to the patient and / or parent or guardian. The Investigator will not suggest further dental treatment based on the result of the images alone.

8.5 Independent Investigator Image Review

After all the images have been taken and verified as acceptable by the originating Investigator, and the CRFs collected, an indpendent review of the Calcivis images will be undertaken to assess the presence or absence of elevated luminescence on the surface to the teeth imaged with the Calcivis System.

This will be carried out by one of the other Investigators as follows:

Site	Original Investigator	Independent Investigator
1	Neil Shanks	Fraser Morrison
1	Elaine Downie	Steve Martin
3	Fraser Morrison	Agnieszka Nohawica
3	Steve Martin	Niel Shanks
5	Agnieszka Nohawica	Elaine Downie

The independent reviewer will be provided with evidence of both the original investigator's decision on tooth classification (i.e. no lesion, or active lesion, based on ICDAS coding / activity status) along with documented evidence of the clinician's intended location for assessment (this is all documented by the original Investigator prior to imaging using the Calcivis System). In addition, photographic evidence of the actual location of the luminescence will be provided. The determination by the independent reviewer will be made off-site, using the visible and Calcivis images along with copies of the original Investigator's documentation. The data from the independent reviewer will be used for the Primary analysis.

9. ADVERSE EVENTS

9.1 Definitions

Adverse Event (AE) – any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device.

This definition includes events related to the investigational device and comparator and events related to the procedures involved.

For users or other persons, this definition is restricted to events related to the investigational medical devices.

A Serious Adverse Event (SAE) – is an adverse event that –

- Led to death
- Led to a serious deterioration in the health of a subject that –
  - resulted in a life-threatening illness or injury
  - resulted in a permanent impairment of a body structure or a body function
  - required in-patient hospitalization or prolongation of existing hospitalization
  - resulted in medical or surgical intervention to prevent a permanent impairment of a body structure or a body function
- Led to foetal distress, foetal death or a congenital abnormality or birth defect

Planned hospitalisation for a pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event

Adverse Device Effect (ADE) – an adverse event related to the use of an investigational medical device.

This definition includes any events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational device.

This definition also includes any event resulting from user error or form intentional misuse of the investigational device.

Serious Adverse Device Effect (SADE) – adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

Anticipated Serious Adverse Device Effect (ASADE) - an effect which by its nature, incidence, severity or outcome has been identified in the risk analysis report

Unanticipated Serious Adverse Device Effect (USADE) - serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk report.

Device Deficiency – inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance.

Device deficiencies include malfunctions, use errors and inadequate labelling.

Device deficiencies which result in SADEs or USADEs, will be managed as detailed below.

Device deficiencies that did not lead to an adverse event, but could have led to a medical occurrence if suitable action had not been taken, or intervention had not been made or if circumstances had been less fortunate will also be managed as detailed below.

Use error – act or omission of an act that results in a different medical device response than intended by the manufacturer or expected by the user.

Use error includes slips, lapses and mistakes.

An unexpected physiological response of the subject does not itself constitute a use error.

Severity Definitions – the following definitions will be used to determine the severity rating for all SAEs

- Mild – awareness of signs or symptoms, that does not interfere with the subject's usual activity, or is transient which resolves without treatment and with no sequelae
- Moderate – a sign or symptom which interferes with the subject's usual activity
- Severe – incapacity with inability to do work or usual activities

9.2 Collection and Reporting of Adverse Events

It is the responsibility of the Investigator at the site to ensure that all adverse events (AEs, SAEs, ADEs, SADEs (ASADEs and USADEs) and device deficiencies) occurring during the course of the study are recorded on the Adverse Event Form. Details recorded should include the following information:

- A description of the event
- The dates of the onset and resolution
- Any action taken
- The outcome
- The relationship to the device
- Whether or not the adverse event is serious
- Whether the adverse events arises from insufficiencies in the IFU
- Whether the adverse event arises from user error

Any adverse events that occur during the course of the study should be treated by established standards of care that will protect the life and health of the study patients.

Adverse events can be observed directly by the site Investigator or staff, or can be spontaneously reported by the patient. In addition each patient should be questioned about adverse events at each study visit. In all cases it is the responsibility of the site Investigator to collect the information and record as outlined above. All adverse events should be followed up for the duration of the study.

In the case of SAEs, SADEs (ASADEs and USADEs) – including those resulting from device deficiencies), these must be reported to the study Sponsor and to the relevant Ethics Committee according to the term of approval and in addition, any local adverse event reporting guidelines should be followed.

Anticipated Adverse Events are as follows:

- Infection due to cross-contamination from other patient
- Irritation / erythema due to reaction to Disclosing Solution
- Hypersensitivity to the Disclosing Solution

- Pain or discomfort due to presence of intra-oral imaging device in mouth
- Damage/ trauma to soft tissue due to presence of intra-oral imaging device in mouth
- Choking hazard from patient biting down on Applicator

Serious Adverse Events, Serious Adverse Device Effects and Unanticipated Adverse Device Effects must be reported to the Sponsor, within 24 hours of becoming aware of the event. In addition a written report is to be provided by the Investigator within 5 working days.

SAEs, SADEs and UDAEs should be sent by email within the specified timelines to:

mwillins@calcivis.com and bvernon@calcivis.com

On receipt of any SAE, SADEs and UADEs, Calcivis Ltd will initiate a Safety Panel review within two working days of becoming aware of the adverse event, with Calcivis Management and Medical / Dental advisors, to determine if there is any safety requirement to stop the clinical study. Any such decision will be communicated to the Investigators as soon as reasonably possible.

10. STATISTICAL ANALYSIS

10.1 Sample size

The study will assess the agreement between the Calcivis System and dentist rating of suitable teeth in two teeth populations: 'no visible lesions', and 'active lesions'. Based on previous study data and expert opinion, 'No visible lesions' are expected to correspond to 'no luminescence' according to the Calcivis system in at least 70% of cases. Similarly, 'active lesions' are also expected to correspond to 'luminescence' in at least 70% of cases.

Subjects will provide no more than one tooth of each tooth population. The study sample size is calculated in terms of the number of teeth of each tooth population required. The number of subjects required is then *at most* the total number of teeth required.

For the purpose of sample size calculations, the percentage agreement for each of the two tooth populations are jointly considered as measures of agreement. That is, the study will be deemed a success if the percentage agreement in both of the tooth status populations is statistically significant at the 2.5% level when compared to chance agreement (50%).

Expressed in terms of hypothesis tests, the null and alternative hypotheses are:

H_0: $p_{a,i}$=0.5 vs. H_1: $p_{a,i}$>0.5 for each tooth status population i

where $p_{a,i}$ is the percentage agreement in tooth status population i.

To achieve at least 90% power overall, a power of 94.9% has been used for each tooth status population individually. The planned method of analysis is an exact binomial test and this has been used to derive a required sample size of 81 for each of the tooth status populations. This is the first sample size after which all subsequent sample sizes provide at least 94.9% power.

10.2 Statistical Analysis

General Considerations

The planned statistical analysis will be fully described in a Statistical Analysis Plan, which will be finalised prior to the locking of the study database. The main details of the planned analysis are described below.

The statistical analysis will be performed using SAS version 9.2 or higher.

All statistical tests will be conducted one-sided with a 2.5% level of significance and no adjustment will be made for multiple testing.

Analysis Populations

All patients on whom the Calcivis System is used will be included in the Safety Population.

The Agreement Population will include all teeth on which there is a dentist ICDAS score.

Analysis

The percentage agreement of the original dentist ICDAS score and the independent reviewer Calcivis finding ('no luminescence' or 'luminescence') will be presented along with an exact one-sided 97.5% confidence interval and p-value comparing the percentage agreement to 0.5 for the following:

- Teeth rated with 'no visible lesion' that are assessed as 'no luminescence' by the Calcivis System;
- Teeth rated with an 'active lesion' that are assessed as 'luminescence' by the Calcivis System.

The analysis of agreement will be performed on the Agreement Population.

The agreement between the original dentist ICDAS score and Calcivis finding will be analysed in the same manner.

User and patient questionnaire data and adverse events will be summarised descriptively for the Safety Population.

Missing Data

Missing data arising from teeth where the Calcivis System assessment is uninterpretable for reasons that are thought unrelated to the assessment outcome, will not be imputed. The reasons will be pre-defined in the Statistical Analysis Plan. Otherwise missing data will be considered as a disagreement in the calculation of agreement. Secondary analyses based on imputing all missing data as a disagreement will be conducted, if appropriate.

Otherwise missing data will not be imputed.

Interim Analysis

No interim analysis is planned for this study.

11. DATA MANAGEMENT

All data collected on the Case Report Forms will be 100% verified against source data (Patient's Dental / Medical Records and Source Document Worksheets) by the monitoring staff. The data from the CRFs will be entered on to a validated Database. Quality control of the data entry process will be performed and any resulting discrepancies adjudicated against the CRFs. The data will then be subjected to validation checks and any resulting Data Queries will be resolved at site with the assistance of the monitoring staff. Once all Data Queries are resolved, critical data will be 100% verified (including adverse events), comparing the Database against the CRF.

The database will then be locked and the study data prepared for statistical analysis.

Any data existing for patients who withdraw voluntarily or are withdrawn from the study, will be used in the study analysis, unless the patient states this is contrary to their wishes.

Details of the data to be presented will be outlined in the Statistical Analysis Plan.

12. STUDY DATA REPORTING AND STUDY REPORT

A final Clinical Study Report of the results will be complied by Calcivis Ltd which will be approved and signed off by each participating Investigator. A copy will be made available to each Investigator. In addition, a lay summary report of the clinical study results will be produced and made available for any patients who request a copy.

Copies of the final Clinical Study Report will be provided to the Research Ethics Committee, NHS R & D and any other local approvers.

13. PUBLICATION OF RESULTS

Calcivis Ltd commits to communicating or otherwise making available for public disclosure the results of the study regardless of the outcome. Public disclosure includes publication of a paper in a scientific journal, abstract submission with a poster or oral presentation at a scientific meeting, or by other means.

At the end of the study, one or more manuscripts for publication will be prepared in collaboration between the all Investigator(s) and Calcivis Ltd on the collective study results. Calcivis Ltd will not suppress or veto publications, however Calcivis Ltd reserves the right to postpone publication and / or communication for 180 days to protect intellectual property.

Any subsequent publications, (journal submissions, posters, white papers, marketing literature etc.) will be covered in a separate Publication Strategy document provided by Calcivis Ltd.

Calcivis Ltd will register this clinical study on www.clinicaltrials.gov website and report the study results accordingly.

14. REGULATORY, ADMINISTRATIVE AND CONTRACTUAL INFORMATION

14.1 Sponsor's Responsibilities

The Sponsor, (Calcivis Ltd) is responsible for providing Investigators with the information and training they need to conduct the clinical study properly and in accordance with the Clinical Study Plan. The Sponsor must ensure proper monitoring of the Clinical Study, that Research Ethics Committee approval, NHS, R & D approval and any other required local approvals are obtained and remain current, and that the Research Ethics Committee, NHS, R & D and other local approvers are informed of significant new information about the clinical study as required.

This information should include the following:

- A current signed copy of the Clinical Study Plan and any amendments
- A signed copy of the signed Clinical Investigation Agreement or equivalent Contract - for each participating Investigator / Site
- All information pertaining to Research Ethics Committee review and approval of this clinical study including a copy of the REC Letter of Favourable Opinion and a blank copy of the approved Patient Information / Consent Form for all sites involved.
- All information pertaining to NHS, R & D or other local approvals / notifications of this clinical study including a copy of the approval letter for each site.
- Copies of current signed and dated Curriculum Vitae's of the Investigator and all relevant site personnel

14.2 Amendments

Any change or addition to this Clinical Study Plan requires a written amendment which must be approved by the Sponsor before the change or addition can be considered effective. Where Research Ethics Committee approval is required, the Principal Investigator must submit the appropriate documentation to the main Research Ethics Committee, and obtain written approval for the amendment before it can be implemented at the investigative site(s). A copy of the written approval must be provided to the Sponsor. All Investigators must submit relevant documentation to each site's NHS, R & D or other relevant local approver and obtain approval before the Amendment can be implemented. Copies of the written approvals must be submitted to the Sponsor. Amendments will be circulated promptly to all investigators by the Sponsor.

14.3 Deviations

A Deviation is a failure to comply with the requirements specified within this Clinical Study Plan without adequate justification. Examples of deviations may include enrolment of a study patient who does not meet all of the inclusion / exclusion criteria specified in the Clinical Study Plan, or missed study visits without adequate documentation.

All deviations must be documented on the appropriate forms and reported to the Sponsor. All deviations will be reviewed and assessed for their impact on patient safety by Calcivis Ltd.

The investigators shall conduct this Clinical Study in accordance with this Clinical Study Plan and any conditions of approval / notification imposed by the Research Ethics Committee, NHS, R & D and / or

other local approvers. Failure to comply with and / or inability to meet these regulations may jeopardise further participation of the Investigator or Investigative Site in this and future clinical studies.

14.4 Monitoring Procedures and Source Documents

A clinical monitor will be appointed by the Sponsor for each investigative site. The monitor is responsible for assessing the Investigator's compliance with the Clinical Study Plan and for performing Source Document Verification. The monitor is also responsible for reporting to the Sponsor on the progress of the Clinical Study.

Source documents include all information, original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study e.g. patient's dental / medical records, dental charts, photographs, patient diaries or questionnaires, device accountability records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, and any certification from medico-technical departments involved in the clinical study.

At the Site Initiation Visit the monitor will review the Clinical Study Plan, Case Report Forms and all associated study documentation and procedures with the Investigator and study personnel. During the course of the study, the monitor will maintain regular contact with the investigative site and conduct on-site monitoring visits and source data verification on a regular basis to ensure compliance with this Clinical Study Plan. The number and frequency of the visits for each site, will be determined by the rate of patient recruitment. During monitoring visits the monitor will require access to the patient's dental / medical records in order to carry out source document verification to ensure all data recorded in the study records is accurate and complete and the data can be submitted to the Sponsor in a timely manner, and to verify that the investigative site facilities continue to be adequate. Throughout the study the monitor must check that all adverse events have been collected, recorded and reported as required and discuss the implication of all Serious Adverse Events with the site Investigator.

The Investigator must set aside a reasonable amount of his / her time for these visits and the time of any relevant site personnel.

14.5 Data Recording

All patients recruited to the study will be identified by a unique study number, comprising the site number and patient number, in order data collected on them will be anonymized. The investigator will maintain a confidential patient identification list of all patients enrolled in the study (by name and patient number). The list will be maintained at the site and will not be retrieved by the Sponsor.

The sites will adhere to all appropriate national and local regulations to protect health information and maintain patient confidentiality.

All evaluations and procedures indicated in this Post-Approval Clinical Study Plan must be performed. All data generated during the course of this Clinical Study will be recorded on standardised 3-part NCR, paper Case Report Forms. CRFs should be completed as soon after the patient visit as possible and only the Principle Investigator may sign and date the designated pages of the CRF for their patients.

14.6 Maintenance, Retention and Archiving of Study Records

Investigators are to maintain all source documents required by regulation, including diagnostic test reports, laboratory results, completed case report forms, supporting medical records and informed consents. The source documents will be referenced during regular monitoring visits to verify the information documented on the case report forms.

The Investigator will retain records for a period of ten years following the date a marketing application is approved for the study device for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for this indication, until ten years after the investigation is discontinued.

Arrangements for archiving of all study documentation will be discussed between the Sponsor and the site.

14.7 Investigator and Site Personnel Training

All key site personnel (Investigators / Dentist and Dental Nurses) must undergo full training on the use of the Calcivis System, Image Interpretation, ICDAS scoring system and Study Procedures as applicable. A record of all training will be maintained.

In addition, Investigators / Dentist and Dental Nurses must undergo GCP training in advance of the Site Initiation Visit unless they have done so already. Such training will be documented.

14.8 Study Termination

The study will be terminated upon completion of follow-up of the last patient recruited. Any decision to either terminate the study early or to increase the patient numbers or follow-up periods will be by mutual agreement between Sponsor, the Investigators and approval by the Research Ethics Committee. Notification will be made of any such decisions to NHS, R & D and other local approvers for each site.

14.9 Financing and Insurance

Financial arrangements will be defined in a Clinical Investigation Agreement or equivalent Contract between Calcivis Ltd and each Investigative site.

Patients and / or parents or guardians will be paid a total Patient Inconvenience Payment (PIP) of £50, to compensate for the time involved in their participation in the study on completion of both study Visits 1 and 2. For patients who have parental / guardian Consent, a payment of £25 in the form of a store voucher or gift card will be made to the patient plus a payment of £25 to the parent or guardian, on completion of both study visits. For all other patients, a payment of £50 will be made on completion of both study Visits 1 and 2. No other payments for expenses will be paid.

Calcivis Ltd as the Sponsor of this Clinical Study will compensate any patient for any injury caused by taking part in this study according to the Association of British HealthCare Industries (ABHI) guidelines. Broadly speaking this means the Sponsor will compensate the patient, without the patient having to prove they are at fault, for any injury as a result of the study device or study procedures. Additional healthcare will be provided for any patients who suffer from an adverse event as a result of participation in this study. The limit for insurance will be £2.5 million.

14.10 Investigator Responsibilities

The Investigator is responsible for ensuring that this clinical study is conducted according to the Clinical Study Plan, the Clinical Investigation Agreement or equivalent Contract, all conditions of appropriate Research Ethics Committee approval, NHS, R & D approval and / or other local approval and any applicable national regulations.

Written confirmation of Research Ethics Committee approval, and any relevant local approvals (e.g. NHS, R & D) must be provided to Calcivis Ltd prior to the enrolment of any subject in the clinical study.

The Investigator is responsible for ensuring that written Informed Consent is obtained from all patients prior to any procedures, tests or treatments that are outside the standard course of treatment that would be followed if this patient were not being considered for enrolment in this clinical study. The Investigator is responsible for informing patients that Calcivis Ltd, and its authorized designees (the study monitor, dental advisor) may have access to their dental / medical records for the purpose of the study. Patients must be informed that they are free to refuse to participate in this clinical study without any impact on their medical treatment and that if they choose to participate, they may withdraw at any time without prejudice to future care. The Research Ethics Committee approved Informed Consent must be signed and dated prior to study participation.

While awaiting for approvals, the Investigator may discuss with a patient their interest in participating in the clinical study, but shall not request the written informed consent nor allow any patient to participate in the clinical study before all relevant approvals are received.

Upon completion of the clinical study or the Investigator's participation in the clinical study, or at the Sponsor's request, the Investigator must return any remaining devices to Calcivis Ltd.

It is the responsibility of the Investigator to maintain complete, accurate and current study records. Each Investigator will be provided with an Investigator Site File, and paper Case Report Forms and other associated study specific documentation by the Sponsor. Such records will be maintained during the course of the clinical study and for ten years following the date on which the study is terminated or completed. Investigator records shall include, but not be limited to the following:

- A current copy of the Clinical Study Plan and any amendments
- A copy of the signed Clinical Investigation Agreement or equivalent Contract
- All information pertaining to Research Ethics Committee Review and approval of this clinical study including a copy of the Research Ethics Committee Letter of Favourable Opinion and a blank copy of the approved Patient Information Sheet and Consent Form on dental practice headed paper.
- All information pertaining to NHS, R & D or other local approvals / notifications of this clinical study including a copy of the approval letter for each site
- Copies of current, signed and dated Curriculum Vitae of the Investigator and all relevant site personnel
- Signed Informed Consent Forms and copies of all completed Case Report Forms and supporting documents (source document worksheets, dental / medical records etc)

- Records of all reports and information pertaining to all serious adverse events
- Accountability records of receipt, use and return of all study devices and other study materials where relevant

15. ETHICAL CONSIDERATIONS

15.1 Standards and Guidelines

This Clinical Study will be performed in accordance with the following standards and guidelines:

- Declaration of Helsinki on Biomedical Research involving Human Subjects (64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013)
- European Standard of BS EN ISO 14155:2011 (E) – clinical Investigation of medical devices for human subjects – Good clinical practice
- International Conference on Harmonisation Good Clinical Practice guidelines (ICH GCP), where applicable

15.2 Research Ethics and other Approvals

Before the study can begin the Investigator must have written evidence of Favourable Opinion for the Clinical Study Plan and associated relevant documentation from the appropriate Research Ethics Committee.

Once approval has been granted, the Investigator is responsible for ensuring that he / she complies with the terms of the approval, namely with adverse event reporting, notification of amendments, interim and final reports on the progress of the study.

Written responses from any other local approvals (e.g. NHS, R & D) must also be obtained prior to starting the study.

15.3 Informed Consent

Written Informed Consent must be obtained from each potential patient or parent or guardian prior to conducting any study assessments or procedures. (In the case of patients aged 6 to 15 years old, written consent must be obtained from the parent or guardian on the patients' behalf, if the patient is not competent to give Written Informed Consent themselves). The Investigator must ensure the nature of the study is fully explained to each patient and / or parent or guardian, and provide the patient and / or parent or guardian with Research Ethics Committee approved copies of the relevant Patient Information Sheet(s) to read. Patient Information Sheets will be provided for patients aged 16 years and older (adults) and for patients aged 12 to 15 years old, a more simplified version will be provided. For patients ages 6 to 11 years, the Patient Information Sheet will be provided in picture format. The patient and / or parent or guardian should be informed that participation in the study is voluntary and by not consenting, it will not affect the patient's right to the most appropriate dental treatment, or affect the dentist / patient relationship. The patient and / or parent or guardian must have adequate time (at least 24 hours) to consider their participation in the study and be able to discuss with others and ask the Investigator any questions. If the patient and / or parent or guardian agrees to participate, a copy of the Research Ethics Committee approved Patient Consent Form must

be signed and dated. A copy of the signed and dated Consent Form (and a copy / copies of the Patient Information Sheets) must be given to the patient and / or parent or guardian to keep. The original signed copy of the Consent Form must be kept by the Investigator and placed in the patient's dental / medical records or scanned in if electronic records are kept. A further copy (or the original) should be placed in the Investigator Site File.

It is understood that informed consent is a matter entirely between the Investigator and the patient and / or parent or guardian. The Sponsor will only confirm that it has been provided; no copy will be taken for use by the company.

Patients and / or parents or guardians are free to withdraw Consent at any time, irrespective of their initial consent. Patients who withdraw Consent will be replaced.

Each patient and / or parent or guardian must also give permission for the Sponsor's representatives to review their dental / medical records for the purpose of source document verification.

During the course of the study, the study patient's details will be kept anonymous (specific study identification codes will be used for each study patient). Study patient data will only be made available to authorized staff of the study Sponsor, it's authorized representatives and regulatory authorities if applicable.

15.4 Disclosure and Confidentiality

By conducting the study, the Investigator agrees that all information provided by the Sponsor will be maintained by the Investigator and the site personnel in strict confidence. It is understood that the confidential information provided to the Investigator will not be disclosed to others without authorization from the Sponsor.

All patients recruited to the study will be identified by a unique site and patient identification number in order data collected on them will be anonymized. The Investigator will maintain a confidential patient identification list of all patients enrolled in the study (by name and patient number). The list will be maintained at the site and will not be retrieved by the Sponsor. Study patient data will only be made available to authorised staff of the Sponsor, it's authorised representatives and regulatory authorities, if applicable.

16. REFERENCES

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18. APPENDICES

Appendix 1 - ICDAS Decision Tree for Caries Codes

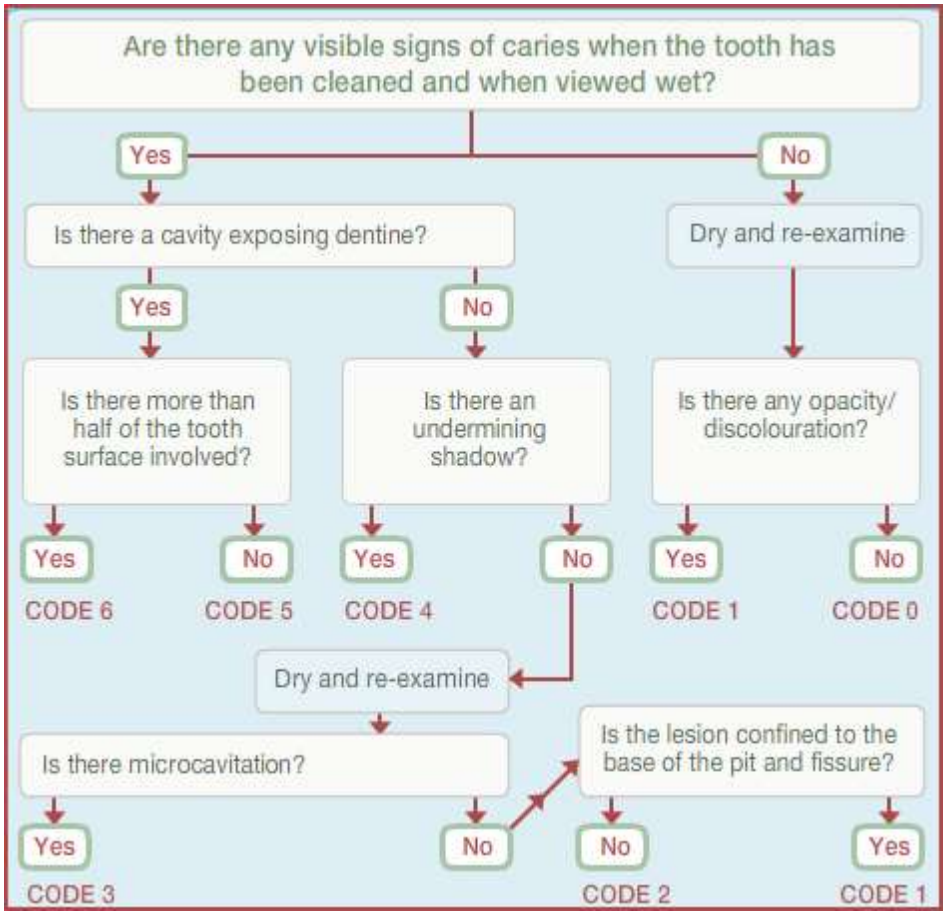
Appendix 2 - Tooth Cleaning Protocol

APPENDIX 1

ICDAS CONVENTIONS – DECISION TREE

ICDAS Decision Tree

Use the decision tree below to help you determine the correct caries code



Caries Codes

0 = Sound tooth surface

1 = First visual change in enamel

2 = Distinct visual change in enamel

3 = Enamel breakdown, no dentine visible

4 = Dentinal shadow (not cavitated into dentine)

5 = Distinct cavity with visible dentine

6 = Extensive distinct cavity with visible dentine

APPENDIX 2

TOOTH CLEANING PROTOCOL

Tooth Cleaning Protocol prior to Imaging with the Calcivis System

Occlusal surfaces:

Remove any dental plaque and debris from the occlusal surface of each molar or premolar which is a potential test site for the Calcivis imaging system using the following protocol:

Use a blunt/ball-ended probe to remove gross accumulations of debris and dental plaque from the occlusal surface, followed by brushing the entire exposed tooth surface with a toothbrush and water (or with a small amount of dental paste at the Investigator's discretion) which has been rotated laterally (horizontally) through 90 degrees to the mesio-distal line of the dental arch and used by approaching the surface from the buccal side, completing a series of vigorous bucco-lingual strokes, together with brush-head rotations. Try to avoid traumatising any remaining operculum present over the occlusal surface.

If dental paste has been used for cleaning, repeat the procedure using tap water only on the toothbrush, in order to remove any remaining prophylaxis paste then thoroughly rinse the occlusal surface with a 3-in-1 (water alone, then air-water spray) to ensure absolutely no prophylaxis paste remains on the occlusal surface prior to the Calcivis imaging. After these procedures, should any particles of prophylaxis paste remain in the pit and fissure pattern of the molar remove these by gently dislodge them with the end of a probe if necessary.

If water only has been used for cleaning, repeat the cleaning with a tooth brush and water only, then thoroughly rinse the occlusal surface with a 3-in-1 (water only, then air-water spray).

Free Smooth surfaces:

Remove any dental plaque and debris from the free smooth surface of each tooth which is a potential test site for the Calcivis imaging system using the following protocol:

Use a blunt/ball-ended probe to remove gross accumulations of debris and dental plaque from the free smooth surface, followed by brushing the entire exposed tooth surface with a toothbrush and water (or with a small amount of dental paste at the Investigator's discretion) which has been rotated laterally (horizontally) through 90 degrees to the mesiodistal line of the dental arch and used by approaching the surface from the appropriatebuccal or lingual/palatal side, completing a series of vigorous cervico-occlusal strokes, together with brush-head rotations. Try to avoid traumatising the gingivae and any remaining operculum present over the occlusal surface of the tooth.

If dental paste has been used for cleaning, repeat the procedure using tap water only on the toothbrush, in order to remove any remaining prophylaxis paste then thoroughly rinse all of the tooth surfaces with a 3-in-1 (water alone, then air-water spray) to ensure absolutely no prophylaxis paste remains on the free smooth surface prior to the Calcivis imaging. After these procedures, should any particles of prophylaxis paste remain in any pit or fissure pattern on the free smooth surface remove these by gently dislodge them with the end of a probe if necessary.

If water only has been used for cleaning, repeat the cleaning with a tooth brush and water only, then rinse thoroughly rinse the free smooth surface with a 3-in-1 (water only, then air-water spray).