CLINICAL STUDY PLAN

Version 5, 08 November 2017

Monitoring of Caries Lesion Activity in Orthodontic Patients with the Calcivis® System –

CAL-03-2015

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Revision History

VERSION / DATE	REVISION CHANGES
Version 1, 19 January 2016	Original Version
Version 2, 01 March 2016	**Page 1 – Front Page)** **Page 3 - Investigator Signature Page)** change of Study Title **Page 7 - 1. Synopsis)** **Footers** amended on every page to reflect amended version and date **Page 9 – 1. Synopsis)** all references to **Page 18 – 5.2 General Description)** needle for re-constitution **Page 21 – 5.6 US Regulatory Status)** removed and replaced by **Page 24 – 5.9 Device Labelling and Storage)** needleless adaptor **Page 24 – 5.9 Device Labelling and Storage –** storage conditions for Disclosing Solution changed from 2 to 8^{0}C to Room Temperature **Page 27 – 8.2 Study Visit 1 –** ethnicity / race removed and orthodontic history clarified **Page 38 – 14.9 – Financing and Insurance** – Patient Inconveneince Payment changed to £20 pro-rata and payments made to patients only
Version 3, 09 November 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 10 – 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 14 – Schedule of Events – Visit 1 de-bond –** addition of Adverse Events collection Pre-imaging and **Visit 4 and Visit 5 –** visit time-windows amended **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 concern and mitigation

	Page 29 – 8.2 Study Visit 1, Page 30 – 8.3 Visit 2 and Page 31 – 8.6 Visit 5 - Preparation of the Calcivis System -clarification of storage conditions for the Calcivis multi-use Disclosing Solution (Photoprotein)
Version 4, 16 May 2017	**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 and title of Principle Investigator changed to Consultant **Page 13 – 1. Synopsis –** **Sites and Investigators** - title of Principle Investigator to Consultant and addition of Investigator – Navin Sinniah **Duration of Procedures and Follow-ups -** number of potential teeth to be imaged reduced from five to four **Page 15 – 2. Schedule of Events –** addition of digital reference photographs at Study Visits 2, 3 and 4 **Page 30 to 33 - Study Visits 1 to 5 –** **Preparation of Calcivis System** – shelf life of reconstituted multi-dose Photoprotein changed from 4 weeks to 2 weeks **Teeth Preparation Procedures -** maximum number of teeth to be imaged changed from five to four and addition of digital reference photographs at Study Visits 2, 3 and 4 **Imaging with the Calcivis device -** maximum number of teeth to be imaged changed from five to four
Version 5, 08 November 2017	**All pages - Footers** - changed on every page to reflect amended version and date **Page 1 – Front Page** – version number and date changed **Page 5 - Investigator Signature Page -** reference to version and date changed in Investigator declaration **Page 10, 14 & 15 – 1. Synopsis –** **Primary Objective –** timeline amended from baseline to 12 weeks post de-bond to baseline to 8 weeks post de-bond **Duration of Procedure and Follow-ups** - follow-up Visits changed from 2, 4, 8 and 12 weeks post de-bond to 4 and 8 weeks post de-bond **Duration of Study** – timelines extended to overall 18 months, with 12 months for recruitment and 3 months for final follow-up, data collection and analysis. **Page 16 – 2. Schedule of Events –** Study Visits amended from Visit 1 (de-bond), Visit 2 (2 weeks post de-bond), Visit 3 (4 weeks post de-bond), Visit 4 (8 weeks post de-bond) and Visit 5 (12 weeks post de-bond) to

	Visit 1 (de-bond), Visit 2 (4 weeks post de-bond) and Visit 3 (8 weeks post de-bond). Patient and User Questionnaires added to final Visit 3 (8 weeks post de-bond) **Page 20 – 4. Study Objectives and Endpoints –** **4.1 Primary Objective -** timeline amended from baseline to 12 weeks post de-bond to baseline to 8 weeks post de-bond **Page 29 & 30 – 7. Study Design** **7.1 – Overview –** overall timeline for follow-up amended from 12 weeks post de-bond to 8 weeks post de-bond. **7.3 Study Duration – t**imelines extended to overall 18 months, with 12 months for recruitment and 3 months for final follow-up, data collection and analysis **Page 30, 32, 33 & 34 – 8. Study Procedures** **8.1 Screening Procedures –** patient availability for the follow-up visits changed from 2, 4, 8 and 12 to 4 and 8 weeks **8.3 Study Visit 2** – changed from 2 to 4 weeks (+/- 3 days) post de-bond **8.4 and 8.5** – removed **8.6** – changed to 8.4 and final study Visit changed from Visit 5 to Visit 3 (+/-5 days) **Post-Imaging Questionnaires –** Visit 5 changed to Visit 3 **8.7 Future Dental Care** -- changed to 8.5 **Page 38 –10.2 Statisitcal Analysis –** **Interim Analysis** - amended to make provision for an Interim Analysis **Page 42 – 14.9 Financing and Insurance –** Patient Inconvenience Payments amended from £20 per visit to £25 per visit

INVESTIGATOR SIGNATURE PAGE

Monitoring of Caries Lesion Activity in Orthodontic Patients with the Calcivis System – CAL-03-2015

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

Print Name: A. CHRISTIE

Signature: _____ Date: 08 NOV 2017

Chief Investigator / Principal Investigator: **Aman Ulhaq MSc. MOrth. MFDS. BDS.**
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I have read this Clinical Study Plan (CAL-03-2015, v5, 08 November 2017) 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: AMAN ULHAQ

Signature: _____ Date: 8/11/17

INVESTIGATOR SIGNATURE PAGE

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Print Name: NAVIN SINNIAH

Signature: _____ Date: 21 NOV 2017

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Monitoring of Caries Lesion Activity in Orthodontic Patients with the Calcivis System – CAL-03-2015

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Print Name: N. McGUINNESS

Signature: _____ Date: 21/11/17

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TABLE OF CONTENTS

1. SYNOPSIS

Study Name and Unique Number	**Monitoring of Caries Lesion Activity in Othodontic Patients with the Calcivis System – CAL-03-2015**
Study Objectives	Primary Objective: Assessment of the Calcivis System to monitor the activity levels of post-orthodontic treatment white spot lesions as an indicator of either 1) gradual arrest of lesion activity (re-mineralisation / repair) or 2) continuing lesion activity (on-going demineralisation), as measured by bioluminescent output over time from baseline (de-bond) to 8 weeks post be-bond (presence or absence of bioluminesence) Secondary Objectives: Assessment of the safety of the Calcivis System, as measured by the collection of all adverse events. Assessment of the value of the feedback / communication with patients after using the Calcivis System, as measured by Questionnaires for both the Patient and the Users

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) • Ampoules of Water for reconstitution • Ampoule Snapper • Syringe • Needleless adaptor (for reconstitution only) **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, single-site, non-randomised, post-approval clinical study

Patient Population:	Eligible patients will be recruited from NHS hospital orthodontic clinics, who have had dental appliances in place for a minimum of 12 months and have visible white spot lesions on the anterior surfaces of incisors and / or canines at de-bond, identified as active with the Caries System.
Inclusion and Exclusion Criteria	<u>Inclusion criteria</u> 1. Patient must be ≥ 12 years old 2. Patient must have had orthodontic appliances placed on the upper incisors and / or canines for at least 12 months, and be ready for de-bond 3. Patient must have at least one, active white spot lesion identified by the Calcivs System immediately post de-bond 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) or within the follow-up period 2. Any patient currently taking part in a clinical research study, or who has taken part in a clinical research study in the previous three months 3. Pregnant and / or nursing mothers
Control	There will be no Control teeth for this study
Number of Patients	Up to 100 patients may have to be recruited, to obtain 45 eligible patients for follow-up

Statistical Rationale	The sample size of 45 patients (with potentially a maximum of five teeth per patient) is not based on statistical power considerations, but as a number of patients that will enable useful data to be collected on the functionality and safety of the Calcivis System for assessing the activity of post-Orthodontic lesions. In addition the data may be used to support the design of future clinical studies. The percentage of teeth showing luminescence using the Calcivis System will be summarised for each post-baseline visit. Additionally the percentage of teeth showing luminescence will be summarised for each post-baseline visit by Investigator and ICDAS score at Visit 1. For each subject the percentage of teeth with each ICDAS score will be calculated. This will then be summarised over all subjects for each post-baseline visit. The ICDAS score will also be cross-tabulated with the presence/absence of luminescence from individual teeth for each visit. User and patient questionnaire data will be summarised descriptively for each visit. Adverse events during the course of the study will also be summarised descriptively. All patients on which the Calcivis System was used at least once will be included in all analyses.
Number of Sites	One (1)

Sites and Investigators	**Site 01:** Mr Aman Ulhaq (Principal Investigator) Consultant – Orthodontics Edinburgh Dental Institute 4th Floor Lauriston Building Lauriston Place Edinburgh, EH3 9HA Scotland Tel: 0131 536 3979 Dr Niall McGuiness Head of Department – Orthodontics Edinburgh Dental Institute 4th Floor Lauriston Building Lauriston Place Edinburgh, EH3 9HA Scotland Tel: 0131 536 3979 Mr Eamon Howard - Bowles Speciality Registrar in Orthodontics Edinburgh Dental Institute 4th Floor Lauriston Building Lauriston Place Edinburgh, EH3 9HA Scotland Tel: 0131 536 3979 Mr Navin Sinniah Postgraduate Student - Orthodontics Edinburgh Dental Institute 4th Floor Lauriston Building Lauriston Place Edinburgh, EH3 9AN Scotland Tel: 0131 536 3979
Duration of Procedure and Follow-ups	Preparation for Imaging is expected to take approximately one minute per tooth with imaging itself taking less than one second per tooth. All eligible teeth (potentially a maximum of four per patient) will be imaged at baseline immediately after de-bond. Teeth with lesions determined active by the Calcivis System at baseline, will be followed up at 4, and 8 weeks post de-bond

Duration of Study	The overall study period is expected to take 18 months – 3 months for Ethics Committee and R & D approval, 12 months for recrutiment and study procedures and 3 months for final follow-up, data collection and analysis.
Regulatory Status	The Calcivis System is a Class IIa Medical Device which is CE marked.

2. SCHEDULE OF EVENTS

	Screening	Visit 1 (de-bond)		Visit 2	Visit 3
	Pre-Imaging	Pre-Imaging	Imaging	4 weeks Post-de-bond (25 to 31 days)	8 weeks Post-de-bond (51 to 61 days)
Identification of suitable patients / issue of Patient Information Sheet	X				
Written Informed Consent		X			
Demographics, Incl. / Excl. criteria		X			
Relevant medical history / meds.		X			
Oral Hygiene information		X			
Orthodontic History		X			
Tooth ID (ICDAS score) and preparation		X		X	X
Reference colour photographs		X		X	X
Calcivis System Imaging			X	X	X
Adverse Events		X	X	X	X
Patient Questionnaire			X		X
User Questionnaire			X		X

3. INTRODUCTION

3.1 Background

Dental caries (tooth decay) is a significant clinical and public health problem. Caries is associated with localised demineralisation 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.

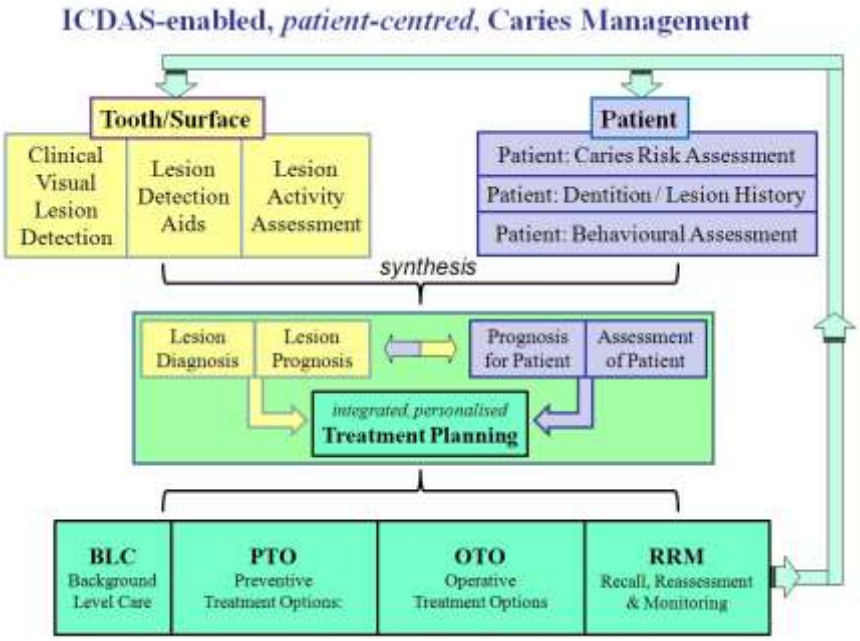
While a number of technologies have been developed to aid caries lesion detection, this step represents only part of the problem for clinicians in relation to clinical decision-making. Determination of the activity status of a lesion is required in order to assess the treatment needs for this lesion. 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 demineralising areas of a tooth surface. The images produced by the system are effectively maps of demineralisation 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 1. 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 has the potential to fall under the Lesion Activity Assessment box, (in yellow with Red border), in figure 1 above. An active lesion is a lesion that is actively deteriorating (demineralisation is outstripping remineralisation). Currently there are no marketed products that allow direct assessment of active demineralisation 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) paint something on it or 3) drill 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

Having previously evaluated that the Calcivis System is both safe and effective, the purpose of this clinical study is to assess the use of the Calcivis System for monitoring the activity levels of post-orthodontic treatment white spot lesions over time, in an Orthodontic setting.

It is generally recognised and reported in the dental literature that patients undergoing orthodontic treatment with fixed appliances find it difficult to maintain meticulous oral hygiene and that, as a result, plaque accumulates adjacent to the orthodontic brackets (which are usually placed on the buccal, Free Smooth, surfaces of teeth, which are readily visible) holding the metal wires which are involved in moving the teeth - thus white spot lesions commonly develop at these sites of plaque stagnation. These lesions are, de facto, actively developing during orthodontic treatment, thus represent 'active lesions'.

Once the wires and brackets are removed post-treatment, normal oral hygiene can be resumed, the plaque/biofilm over these lesions is readily removed and they become arrested, i.e. the demineralisation process ceases. Thus during a short period of time – a matter of weeks – there is usually a transition from active to inactive status for these 'orthodontically-induced' white spot lesions. By assessing and monitoring these lesions, using the Calcivis device, at relatively short intervals during this period of lesion status transition immediately post orthodontic treatment it would be possible to monitor the reduction in the demineralisation process as these lesions arrest.

4. STUDY OBJECTIVES AND ENDPOINTS

4.1 Primary Objective

Primary Objective - to assess the Calcivis System for monitoring the activity levels of post-orthodontic treatment white spot lesions as an indicator of either 1) gradual arrest of lesion activity (remineralisation / repair) or 2) continuing lesion activity (on-going demineralisation) of the Calcivis System –

this will be measured by bioluminescent output over time from baseline (de-bond) to 8 weeks post be-bond (presence or absence of bioluminesence)

4.2 Secondary Objectives

Secondary Objectives

To assess the safety of the Calcivis System –

this will be measured by the collection of all adverse events.

To assess the value of feedback and/or communication with patients after using the Calcivis System –

this will be measured by Questionnaires for both the Patient and the User

5. STUDY DEVICE

5.1 Intended Use and Indications

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 accessible 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:

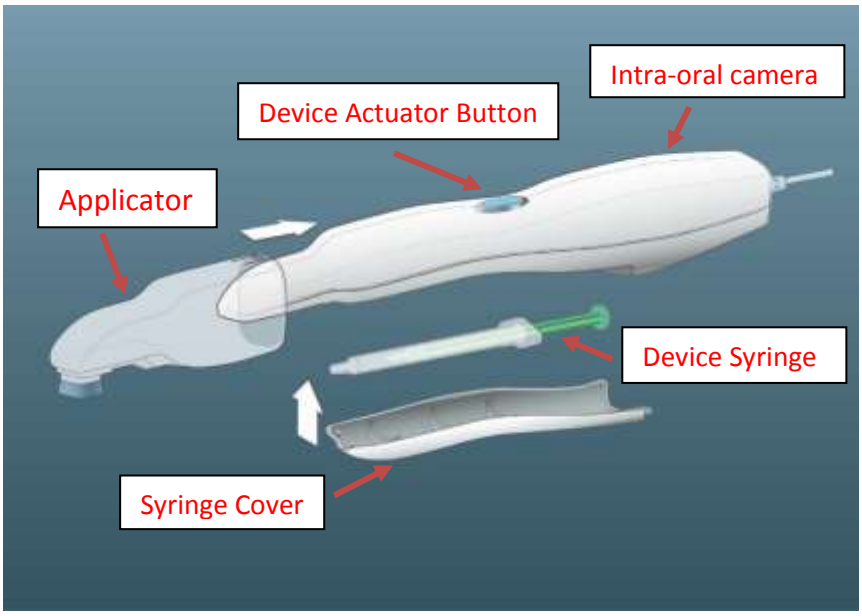
Calcivis Imaging Kit - Administration and Imaging device
Consists of:

- 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)
- Ampoules of Water for reconstitution
- Ampoule Snapper
- Syringe
- Needleless adaptor (for reconstitution only)

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 (wter 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).

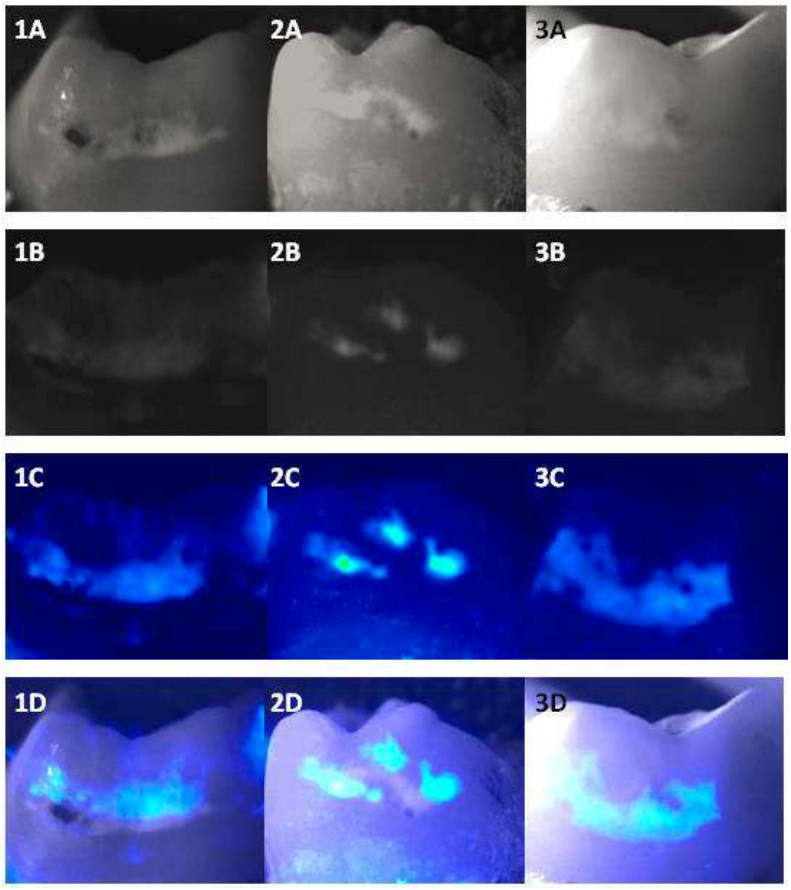
Full details for the operation of the Calcivis System is supplied in the Instructions For Use.

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 25µl 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 needleless

Class I Rule 5 invasive (via natural orifice). Syringe and needleless adaptor 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 in to 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 luminescence, (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 Investigator 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 in non-sterile boxes, each box containing 10 vials each of the freeze-dried protein and water for reconstitution. In addition each box will contain a snapper for safe opening of the vials. Each vial will be identified by a unique lot number.

The Application Kits will be supplied in boxes of ten, each kit containing an individually wrapped applicator, an individually wrapped, sterile, off- the- shelf syringe, and an individually wrapped, sterile, off- the- shelf needless adaptor. The individual Applicators will be identified by a unique lot number.

The Calcivis multi-use format will be supplied in a non-sterile 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 Intra-Oral Imaging Device and Application Kits will be stored at room temperature, and will be held in a secure area at site when not in use. The Disclosing Solution Kits, containing the lyophilised protein and Calcivis diluent for reconstitution, will be stored in a secure area not above 25^0 C. For the Calcivis multi-use format, once reconstituted the vial of Photoprotein will be stored at 2 to 8^0 C, up to a maximum of 4 weeks.

5.10 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 coding system
- Tooth cleaning protocol
- Full preparation and use of the Calcivis Sytem according to the manufacturer's Instructions For Use, to include –
    - preparation of the disclosing solution, syringe loading and device activation
    - laptop set-up, image interpretation and storage
- Informed Consent procedures, study procedures and data recording
- Adverse event collection, recording and reporting

All training will be fully documented for Investigators / Orthodontists 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. Extensive 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 evaluate the Calcivis System for monitoring lesion activity behaviour in orthodontic patients by evaluating active demineralization on tooth surfaces over time, in a clinical setting. When used to characterise and monitor 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 lesons 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, single-site, non-randomised, post-approval clinical study to assess the use of the Calcivis System for monitoring the activity levels of post-orthodontic treatment white spot caries lesions over time, from baseline (de-bond) to 8 weeks post de-bond.

This post-approval clinical study will be conducted under the controlled conditions of this clinical study plan, on eligible patients in a clinical setting (NHS hospital, orthodontic clinic), by three Investigators.

7.2 Patient Selection and Confidentiality

Patients will be recruited to the study from an NHS hospital, orthodontic clinic. Patients will initially be identified and selected according to Inclusion Criteria and the Exclusion Criteria listed below from those attending for orthodontic treatment and who are ready for de-bond. Patients must provide written informed consent (or parents or guardians where the patient is not competent to provide written informed Consent), and be willing to adhere to the study schedule, before being entered in to the study, however, only patients who have 'active' lesions on de-bond, as confirmed with the Calcivis System at the baseline visit, will be followed up in this study. If all lesions are assessed as 'inactive', the patient will be withdrawn from the study.

It is expected that up to 100 patients may have to be recruited and imaged with the Calcivis System at de-bond (baseline), in order to find 45 patients with active lesions.

Patients will be encouraged to complete all 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 anonymised. 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.

Only patients with 'active' lesions will be followed up in this study, therefore no randomization will be required.

7.3 Study Duration

The overall study period is expected to take 18 months - 3 months for Ethics Committee and NHS, R & D approvals, 12 months for recruitment and study procedures, and 3 months for final follow up, data collection and analysis.

7.4 Inclusion and Exclusion criteria

Inclusion criteria

1. Patient must be ≥12 years old
2. Patient must have had orthodontic appliances placed on the upper incisors and canines for at least 12 months, and be ready for de-bond
3. Patient must have at least one, active white spot lesion identified by the Calcivis System immediately post de-bond
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

Exclusion criteria

1. Any Patient with recent tooth bleaching (within previous two weeks of imaging with the Calcivis System or within the follow-up period)
2. Any patient currently taking part in a clinical research study, or has taken part in a clinical research study in the previous three months
3. Pregnant and / or nursing mothers

8. STUDY PROCEDURES

8.1 Screening Procedures

Patients attending routine Orthodontic appointments to have their appliances checked, and who are identified by the Investigator as meeting the Inclusion and Exclusion criteria, and are ready for de-bond, will be approached to discuss their possible participation in the study.

Initial approach will be made 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 Patient Information Sheet and Consent Forms to take away to read and discuss with others if required. Patients and / or parent or guardian will be asked to confirm their interest in participating in the study by contacting the Orthodontic clinic 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 and to ask the Investigator 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 Orthodontic clinic for Study Visit 1 (baseline), when written Informed Consent will be taken by the Investigator. Patient availability for the follow-up visits at 4 weeks (+/- 3 days) and 8 weeks (+/- 5 days) will also be checked.

The Site will document all patients and / or parents or guardians provided with the Patient Information Sheets, those attending Visit 1 baseleine Visit and the outcome on a Patient Identification / Recuitment Log.

8.2 Study Visit 1 – de-bond - baseline

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

Pre-Imaging Information collected

Demographics – DoB and gender

Relevent medications – calcium supplements and antacids

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

Orthodontic history – date braces applied, type of bracket, ligation used, and any other relevant information (e.g. re-mineralization products used)

Preparation of the Calcivis System

The Calcivis System must be prepared according to the manufacturer's Instructions For Use. Single-use Disclosing Solution (Photoprotein) should not be reconsituted 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 8^0, up to a maximum of 2 weeks.

Teeth Preparation Procedure

Immediately after the applicances have been removed, up to a maximum of four teeth from any of the four upper incisors and two canines with visible white spot lesions on de-bond, will be identified and recorded for assessment using the Calcivis System.

Each free smooth surface will be air dried before taking close-up colour photographs of the tooth or teeth with a digital camera for reference, and coded for ICDAS (1,2 or 3).

All relevant teeth will then be cleaned by the Orthodontist, by brushing with water, 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.

Imaging with the Calcivis device

Extreme care must be taken to ensure the surface of the tooth and surrounding area are free from saliva before imaging with the Calcivis System by air drying for a minimum of 10 seconds immediately before application of the disclosing solution and Calcivis System imaging.

Images of the free smooth tooth surfaces, will be taken with the Calcivis System according the Instructions For Use. If the first image of a selected tooth is not clear, it is acceptable to take a second image of the selected tooth, however patients must rinse out with water before the subsequent image

can be taken, and the tooth air-dried as described above. A maximum of four images per patient can be taken with the Calcivis System.

Immediately 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.

The images generated with the Calcivis System will be stored digitally on the laptop provided. The software overlays the two sets of images (before and after application of the disclosing solution), resulting in a demineralization map of each imaged tooth.

At the end of the imaging procedures, the Investigator will review the images on the laptop and record any 'activity' as a YES or NO, according to areas of elevated bioluminescence. If there is no area of elevated bioluminescence on a selected tooth surface, that tooth will not be followed up at future visits. Only patients with at least one tooth surface showing areas of active demineralization will be followed up.

In the event that none of the teeth imaged from a patient show any active demineralization, that patient will be withdrawn from the study and will be replaced.

At the end of Visit 1, the Investigator will share the images of the teeth with the patient and discuss the results.

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

Representatives of the Sponsor, Calcivis Ltd, will be available throughout Visit 1 and any subsequent visits, 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.

Patients will then resume routine procedures carried out at de-bond. Patients will be advised to follow a routine tooth brushing / oral hygiene regime of brushing teeth at least twice per day with a fluoride toothpaste, taking care to brush any areas highlighted by the orthodontist, and to use a mouthwash at different times of the day from brushing.

Post-Imaging Questionnaires

At the end of the imaging procedures, patients will be asked to remain at the Orthodontic clinic to complete a Patient Questionnaire. Visit 1 is then complete.

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

8.3 Study Visit 2 – 4 weeks post-de-bond (+ / - 3 days)

Preparation of the Calcivis System

The Calcivis System must be prepared according to the manufacturer's Instructions For Use. Single-use Disclosing Solution (Photoprotein) should not be drawn up in to the syringe more than 2 hours before

the first tooth is imaged. Once reconstituted, the multi-use vial of Disclsoing Solution (Photoprotein) should be stored at 2 to 8^0 C, up to a maximum of 2 weeks.

Teeth Preparation Procedures

All teeth found to be active at Visit 1, will be cleaned by the Orthodontist, by brushing with water, 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. Each free smooth surface will be air dried before being coded for ICDAS (0, 1, 2 or 3).

Close-up digital photographs will be taken of the selected teeth for reference (as per routine orthdontic procedures).

Imaging with the Calcivis device

Extreme care must be taken to ensure the surface of the tooth and surrounding area are free from saliva before imaging with the Calcivis System by air drying for a minimum of 10 seconds immediately before application of the disclosing solution and Calcivis System imaging.

Images of the free smooth tooth surfaces, will be taken with the Calcivis System according to the Instructions For Use. If the first image of a selected tooth is not clear, it is acceptable to take a second image of the selected tooth, however patients must rinse out with water before the subsequent image can be taken, and the tooth air-dried as described above. A maximum of – four images per patient can be taken with the Calcivis System.

Immediately 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.

At the end of the imaging procedures, the Investigator will review the images on the laptop and record any “activity” as a YES or NO, according to areas of elevated bioluminescence

The Investigator will share the images of the teeth with the patient at the end of the Visit.

Patients will be advised to follow a routine tooth brushing / oral hygiene regime of brushing teeth at least twice per day with a fluoride toothpaste, taking care to brush any areas highlighted by the orthodontist, and to use a mouthwash at different times of the day from brushing.

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

Visit 2 is then complete.

8.4 Study Visit 3 – 8 weeks post-de-bond (+ /- 5 days) – final study visit

Preparation of the Calcivis System

The Calcivis System must be prepared according to the manufacturer’s Instructions For Use. Single-use Disclosing Solution (Photoprotein) should not be drawn up in to the syringe 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 8^0 C, up to a maximum of 2 weeks.

Teeth Preparation Procedures

All teeth found to be active at Visit 1, will be cleaned by the Orthodontist, by brushing with water, 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. Each free smooth surface will be air dried before taking close-up colour photographs of the tooth or teeth with a digital camera for reference, and coded for ICDAS (0, 1, 2 or 3). Imaging with the Calcivis device

Extreme care must be taken to ensure the surface of the tooth and surrounding area are free from saliva before imaging with the Calcivis System by air drying for a minimum of 10 seconds immediately before application of the disclosing solution and Calcivis System imaging.

Images of the free smooth tooth surfaces, will be taken with the Calcivis System according the Instructions For Use. If the first image of a selected tooth is not clear, it is acceptable to take a second image of the selected tooth, however patients must rinse out with water before the subsequent image can be taken, and the tooth air-dried as described above. A maximum of four images per patient can be taken with the Calcivis System.

Immediately 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.

At the end of the imaging procedures, the Investigator will review the images on the laptop and record any “activity” as a YES or NO, according to areas of elevated bioluminescence

The Investigator will share the images of the teeth with the patient at the end of the Visit.

Patients will be advised to follow a routine tooth brushing / oral hygiene regime of brushing teeth at least twice per day with a fluoride toothpaste, taking care to brush any areas highlighted by the orthodontist, and to use a mouthwash at different times of the day from brushing.

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

Post-Imaging Questionnaires

At the end of the imaging procedures, patients will be asked to remain at the Orthodontic clinic to complete a Patient Questionnaire. Visit 3 is then complete.

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

Anonymised images from the study patients may be reviewed at a later date by representatives of the Sponsor for publications, educational and / or promotional use. Consent will be sought from the patient and / or parent or guardian.

8.7 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.

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

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 sample size of 45 patients (with potentially a maximum of five teeth per patient) is not based on statistical power considerations, but as a number of patients that will enable useful data to be collected on the functionality and safety of the Calcivis System for assessing the activity of post-Orthodontic lesions. In addition the data may be used to support the design of future clinical studies.

Therefore, it is intended to recruit a total of 45 patients to the study, who have at least one tooth with a visible caries lesion on debond who will be followed up for 12 weeks.

It is expected that up to 100 patients may have to be recruited and imaged with the Calcivis System at de-bond (baseline), in order to find 45 patients with active lesions.

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

Analysis Populations

All patients on whom the Calcivis System is used will be included in the Safety Population. This population will be used for all summaries.

For each subject the percentage of teeth showing luminescence using the Calcivis System will be calculated. This will then be summarised over all subjects for each post-baseline visit. Additionally the percentage of teeth showing luminescence will be summarised for each post-baseline visit by Investigator and ICDAS score at Visit 1.

For each subject the percentage of teeth with each ICDAS score will be calculated. This will then be summarised over all subjects for each post-baseline visit. The ICDAS score will also be cross-tabulated with the presence / absence of luminescence from individual teeth for each visit.

User and Patient questionnaires will be summarised descriptively for each visit.

Adverse events during the course of the study will also be summarised descriptively.

Missing Data

Missing data will not be imputed.

Interim Analysis

An interim report of the study may be produced before all patients have completed the study. Since there is no statistical testing being performed, no adjustments to the statistical analysis are required due to any interim report.

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 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 REC, 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, clinical charts, laboratory reports, 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 the patient 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 anonymysed. 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 at each site may sign and date the designated pages of the CRF.

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 dental / 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 preparation, set-up and use of the Calcivis System, Image Interpretation, ICDAS scoring system and Study Proecdures as applicable. A record of <u>all</u> 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 will be paid a Patient Inconvenience Payment (PIP) of £25 at the end of each study visit attended and completed (a total of £75 for all three study visits) , to compensate for the time involved in their participation in the study. 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 REC 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 REC Letter of Favourable Opinion and a blank copy of the approved Patient Information Sheet and Consent Form on hospital 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 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 mateirals 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)

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 12 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 Reasearch 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. 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 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.

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 anonymysed. 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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11. Alves LS, Zenker JEA, Wagner MB, Dame-Teixeira N, Susin C and Maltz M. Eruption Stage of Permanent Molars and Occlusal Caries Activity / Arrest – JDR Clinical Research Supplement, July 2014, p114S-118S.

17. APPENDICES

Appendix 1 - ICDAS Decision Tree for Caries Codes

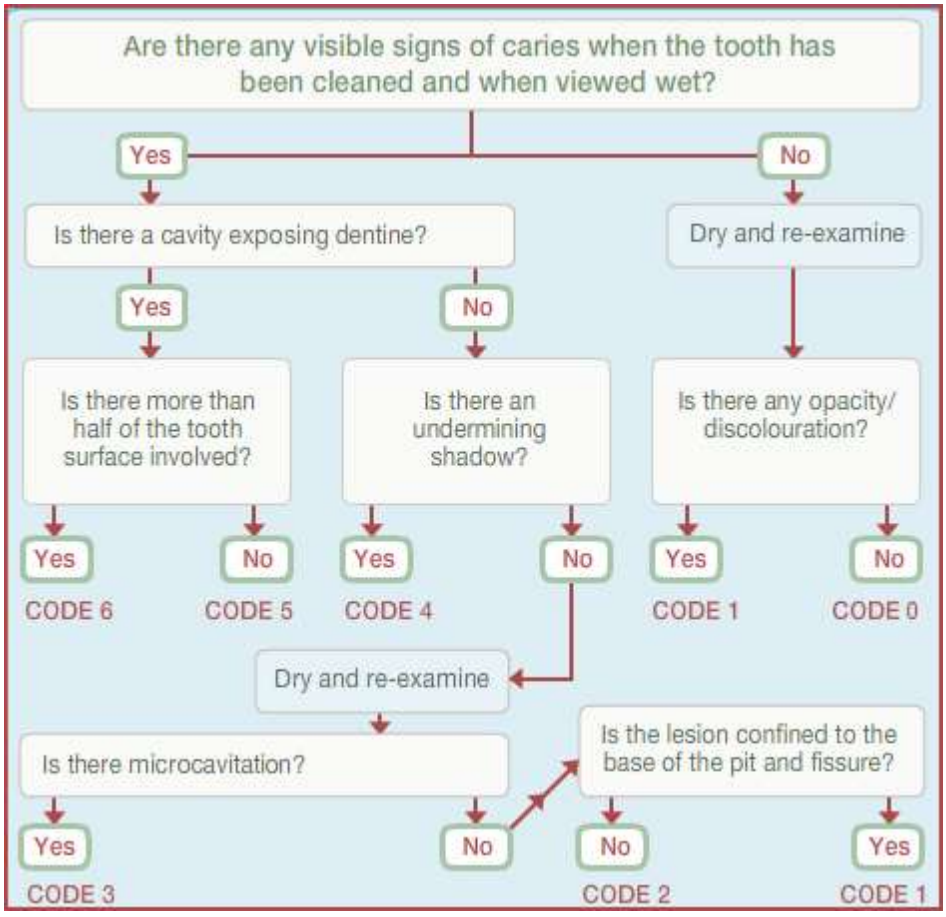
Appendix 2 – Tooth Cleaning Protocol

APPENDIX 1

ICDAS CONVENTIONS

ICDAS Decision Tree for Caries Codes

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

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), and aiming to avoid traumatising the gingivae.

Follow this cleaning by thoroughly rinsing all of the tooth surfaces with a 3-in-1 (water alone, then air-water spray) to ensure absolutely no gross accumulations of debris and dental plaque remains on the free smooth surface prior to the Calcivis imaging.