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Abstract

Accuracy of fundus autofluorescence imaging for the diagnosis and monitoring of retinal conditions: a systematic review

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Background: Natural fluorescence in the eye may be increased or decreased by diseases that affect the retina. Imaging methods based on confocal scanning laser ophthalmoscopy (cSLO) can detect this 'fundus autofluorescence' (FAF) by illuminating the retina using a specific light 'excitation wavelength'. FAF imaging could assist the diagnosis or monitoring of retinal conditions. However, the accuracy of the method for diagnosis or monitoring is unclear.

Objective: To conduct a systematic review to determine the accuracy of FAF imaging using cSLO for the diagnosis or monitoring of retinal conditions, including monitoring of response to therapy.

Data sources: Electronic bibliographic databases; scrutiny of reference lists of included studies and relevant systematic reviews; and searches of internet pages of relevant organisations, meetings and trial registries. Databases included MEDLINE, EMBASE, The Cochrane Library, Web of Science and the Medion database of diagnostic accuracy studies. Searches covered 1990 to November 2014 and were limited to the English language.

Review methods: References were screened for relevance using prespecified inclusion criteria to capture a broad range of retinal conditions. Two reviewers assessed titles and abstracts independently. Full-text versions of relevant records were retrieved and screened by one reviewer and checked by a second. Data were extracted and critically appraised using the Quality Assessment of Diagnostic Accuracy Studies criteria (QUADAS) for assessing risk of bias in test accuracy studies by one reviewer and checked by a second. At all stages any reviewer disagreement was resolved through discussion or arbitration by a third reviewer.

Results: Eight primary research studies have investigated the diagnostic accuracy of FAF imaging in retinal conditions: choroidal neovascularisation (one study), reticular pseudodrusen (three studies), cystoid macular oedema (two studies) and diabetic macular oedema (two studies). Sensitivity of FAF imaging using an excitation wavelength of 488 nm was generally high (range 81–100%), but was lower (55% and 32%) in two studies using longer excitation wavelengths (514 nm and 790 nm, respectively). Specificity ranged from 34% to 100%. However, owing to limitations of the data, none of the studies provide conclusive evidence of the diagnostic accuracy of FAF imaging.

Limitations: No studies on the accuracy of FAF imaging for monitoring the progression of retinal conditions or response to therapy were identified. Owing to study heterogeneity, pooling of diagnostic outcomes in meta-analysis was not conducted. All included studies had high risk of bias. In most studies the patient spectrum was not reflective of those who would present in clinical practice and no studies adequately reported how FAF images were interpreted.

Conclusions: Although already in use in clinical practice, it is unclear whether or not FAF imaging is accurate, and whether or not it is applied and interpreted consistently for the diagnosis and/or monitoring of retinal conditions. Well-designed prospective primary research studies, which conform to the paradigm of diagnostic test accuracy assessment, are required to investigate the accuracy of FAF imaging in diagnosis and monitoring of inherited retinal dystrophies, early age-related macular degeneration, geographic atrophy and central serous chorioretinopathy.

Study registration: This study is registered as PROSPERO CRD42014014997.

Funding: The National Institute for Health Research Health Technology Assessment programme.

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List of abbreviations

AF	autofluorescence
AMD	age-related macular degeneration
CBR	confocal blue reflectance
CFP	colour fundus photography
CMO	cystoid macular oedema
CSC	central serous chorioretinopathy
cSLO	confocal scanning laser ophthalmoscopy
DMO	diabetic macular oedema
DR	diabetic retinopathy
FA	fluorescein angiography
FAF	fundus autofluorescence
GA	geographic atrophy
ICGA	indocyanine green angiography
IR	infrared reflectance
MPOD	macular pigment optical density
NIR-FAF	near-infrared fundus autofluorescence
NPV	negative predictive value
OCT	optical coherence tomography
PPV	positive predictive value
QUADAS	Quality Assessment of Diagnostic Accuracy Studies instrument
RF	red-free (photography)
RM-SLO	retromode scanning laser ophthalmoscopy
RNIB	Royal National Institute of Blind People
RPD	reticular pseudodrusen
RPE	retinal pigment epithelium
SD-OCT	spectral domain optical coherence tomography
SLO	scanning laser ophthalmoscopy
TD-OCT	time domain optical coherence tomography

Plain English summary

The retina of the eye (where light is detected) has a natural faint glow called 'autofluorescence' which can be seen using a specialised camera. Diseases of the retina can affect the intensity of this autofluorescence. As such, measuring autofluorescence of the retina (called 'autofluorescence imaging') could help in diagnosing retinal diseases, or in monitoring their progression or response to treatment. However, the accuracy of autofluorescence imaging for these purposes is unknown. We conducted a rigorous systematic review of research studies to clarify the diagnostic and monitoring accuracy of autofluorescence imaging. Eight relevant studies were found but they had investigated only the diagnosis, not the monitoring, of retinal diseases. Four of these studies were diagnosing different aspects of a condition called age-related macular degeneration and the other four were diagnosing different types of swelling (oedema) of the retina. All eight studies have limitations in their methods, which means that their results may not be reliable and are unlikely to be relevant to real-world clinical practice. Therefore, it is unclear whether or not autofluorescence imaging would be accurate for diagnosing or monitoring retinal diseases in clinical practice. Based on the available evidence, we provide structured recommendations for future research. There is a need for studies that are relevant to actual clinical practice, with patients similar to those who would be tested in real life and that involve comparison tests that are relevant. Studies would be helpful in diseases where autofluorescence imaging appears most useful for diagnosis and/or monitoring. These include inherited retinal diseases and 'geographic atrophy'.

Scientific summary

Background

Retinal conditions are diseases associated with the retina, that is, the part of the eye that collects light and converts it into electrical signals. They include, among others, age-related macular degeneration (AMD), central serous chorioretinopathy (CSC), inherited retinal dystrophies, diabetic retinopathy and cystoid macular oedema. Early identification of retinal conditions and disease stage is essential to allow prompt diagnosis, enabling timely treatment to prevent visual loss for treatable conditions such as neovascular AMD. However, for many retinal conditions it may be possible to reduce only the symptoms or slow disease progression, which can prolong the time during which affected people can continue their normal activities. Information about diagnosis is also important for patients, particularly regarding the prognosis and genetic risks of inherited eye disease. Developments in imaging techniques, particularly with the evolution of scanning laser ophthalmoscopes, have enabled more detailed inspection of the retina and provided less invasive tools to guide treatment and monitor the efficacy and safety of treatments. At the same time, advances in treatments for retinal conditions have increased the need for more accurate information on differential diagnosis and prognosis, so that treatment can be appropriately targeted. Fundus autofluorescence (FAF) imaging, based on scanning laser ophthalmoscopy, is a relatively new method that assesses retinal health by detecting changes in the natural fluorescence of the retina. The presence, absence and intensity of FAF can be affected by diseases of the retina, meaning that FAF imaging could aid in the diagnosis and/or monitoring of retinal conditions. However, the accuracy of the method for diagnosing and monitoring different retinal conditions is unclear.

Objectives

The aim of this project was to assess the accuracy of FAF imaging using confocal scanning laser ophthalmoscopy (cSLO) for the diagnosis and monitoring of retinal conditions. Specific research objectives were:

- for each retinal condition, to determine the diagnostic and monitoring accuracy of FAF imaging using cSLO, including monitoring of disease management
- to identify future research needs and develop research recommendations.

Methods

A review of evidence for the diagnostic and monitoring accuracy of FAF imaging for retinal conditions was undertaken systematically based on pre-specified inclusion criteria. Patients with any retinal condition were eligible, except malignancy, other ocular disease (e.g. glaucoma), or retinal trauma. Electronic databases searched included MEDLINE (via Ovid); MEDLINE In-Process & Other Non-Indexed Citations; EMBASE; The Cochrane Library; Web of Science; Database of Abstracts of Reviews of Effects; Health Technology Assessment database; and the Medion database of diagnostic accuracy studies. Internet pages of relevant organisations and meeting and trial registries were also searched, and reference lists of included studies and relevant systematic reviews were checked. All databases were searched from 1990 (approximately 10 years prior to the likely publication of the earliest relevant evidence) to November 2014 and searches were limited to the English language. The evidence synthesis and analysis followed good practice approaches, as recommended by the Centre for Reviews and Dissemination and the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy. Two reviewers independently screened the titles and abstracts of all bibliographic records identified against prespecified inclusion criteria. Full-text records were

obtained for those titles and abstracts that either appeared to meet the inclusion criteria or for which relevance was unclear, and these were screened against the prespecified eligibility criteria by one reviewer and checked by a second reviewer. Extraction of data from included studies was undertaken by one reviewer and checked by a second. Two reviewers independently assessed the risk of bias of the diagnostic studies using a modified version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) instrument. At all stages of the review, any disagreement between the two reviewers was resolved through discussion or, if necessary, arbitration by a third reviewer. Synthesis of included studies consisted of a structured narrative with tabulation of results. An advisory group comprising two independent clinical experts and a representative of a national charity supporting people with sight problems informed the review.

Results

Number and quality of studies

Searches identified 2240 bibliographic records, from which 206 full-text papers were obtained for further inspection; eight full-text papers reporting eight primary research studies were included in the systematic review. These eight studies all reported diagnostic accuracy of FAF imaging. No studies on the accuracy of FAF imaging for monitoring retinal conditions (i.e. monitoring progression or response to therapy) met the inclusion criteria. The diagnostic accuracy of FAF imaging was reported for choroidal neovascularisation in neovascular AMD in one study; for reticular pseudodrusen in three studies [in early AMD, geographic atrophy (GA) or neovascular AMD]; for cystoid macular oedema secondary to various conditions in two studies; and for diabetic macular oedema in two studies. The included studies have a number of limitations when assessed against the QUADAS criteria. Notably, the studies were considered to be at high (or in one case unclear) risk of spectrum bias (i.e. the patient population would not be representative of people presenting for retinal imaging in current NHS practice) and there are uncertainties around the relevance of the reference standards in most of the studies. Although the reference standards were not necessarily inappropriate, they were all single imaging tests, whereas in clinical practice diagnosis would more likely be based on combined information from several tests. In all studies the risk of clinical review bias was deemed unclear, as the information required to interpret FAF images was not reported.

Diagnostic outcomes

Meta-analysis of sensitivity and specificity of FAF imaging was considered inappropriate owing to the heterogeneity of the study populations, as well as the limited number of studies available for each retinal condition. Most included studies used an excitation wavelength of 488 nm and reported high sensitivity of FAF imaging (range 81–100%). However, sensitivity was lower in two studies that used longer excitation wavelengths: 32% in a study of reticular pseudodrusen in AMD using 790 nm; and 55% in a study of diabetic macular oedema using 514 nm. The specificity of FAF imaging across all studies ranged from 34% to 100% and was not clearly related to the excitation wavelength. However, owing to the relative paucity of reliable data, and limitations in experimental rigour, these diagnostic outcomes are subject to considerable uncertainty and may not accurately reflect the diagnostic accuracy of FAF imaging when applied in clinical practice. As such, none of the eight primary studies provides conclusive quantitative evidence for the diagnostic accuracy of FAF imaging in any of the four retinal conditions they examined. More robust studies would be helpful to quantify test accuracy and these should ideally be conducted to address clinical scenarios relevant to current NHS practice. There is currently no information available on the diagnostic or monitoring accuracy of FAF imaging for inherited retinal dystrophies (such as retinitis pigmentosa, Stargardt disease and rod–cone dystrophies), early AMD, AMD-related GA or CSC. These conditions were identified by the review advisory group as being where FAF imaging might potentially be most useful for assisting diagnosis or monitoring disease progression in NHS practice.

Discussion

Strengths of the evidence synthesis

The current review is based on a prespecified, peer-reviewed protocol. It included comprehensive literature searches in a wide variety of data sources undertaken by an experienced information specialist. The study selection and data extraction steps were pilot-tested and are based on prespecified worksheets, which are provided as appendices to this report. The primary evidence was assessed using prespecified and internationally accepted critical appraisal criteria for test accuracy studies. All studies excluded at the full-text screening step are listed in an appendix, stating the reasons for exclusion. All steps of the systematic review were carried out by at least two reviewers, to minimise the risks of errors and bias. An independent advisory group informed the review.

Limitations of the evidence synthesis

Interpretation of the primary research is hampered by clinical heterogeneity among the included studies and limitations in their methodological rigour. In some cases where studies included both eyes of patients in the analysis, intrasubject correlations may have led to underestimation of standard errors for diagnostic outcomes. This was not assessed quantitatively; however, it would not have markedly affected the overall conclusions. As prespecified in the protocol, searches were limited to evidence published in the English language.

Uncertainties

The extent of use of FAF imaging for diagnosing and/or monitoring retinal conditions in the NHS is not generally known, although the project's advisory group suggested specialists in the field of inherited retinal degeneration might already use FAF imaging routinely. The diagnostic accuracy of FAF imaging has been assessed only in primary research studies on four retinal conditions, and it remains unclear whether or not the technique would accurately diagnose other conditions, including the inherited retinal dystrophies, early AMD, GA and CSC. Numerous studies have monitored qualitatively the progression of retinal conditions or their response to therapy using FAF imaging, but it is unclear whether or not FAF imaging is accurate as a monitoring tool since no studies have formally assessed this quantitatively. A key limitation of the included studies is that none reported the clinical information necessary to interpret the FAF images, so it is unclear whether or not the interpretation in the studies would be consistent with how FAF images are interpreted in clinical practice.

Conclusions

It is not possible to give a clear indication of the diagnostic or monitoring accuracy of FAF imaging for retinal conditions based on existing research, even though FAF imaging appears to be already used in the NHS for diagnosing certain retinal conditions. Although some studies reported relatively high diagnostic sensitivity, these had various methodological limitations that hinder the interpretation of test accuracy. There is an indication that standard wavelength FAF imaging (488 nm) may be more sensitive than longer-wavelength approaches, but this is based on only two studies, involving 790-nm imaging for detecting reticular pseudodrusen and 514-nm imaging for detecting diabetic macular oedema. Owing to the relative paucity of reliable data, further studies are required. In particular, prospective studies are required in inherited retinal dystrophies, dry AMD, GA and CSC, and the studies should be designed according to the paradigm for the quantitative assessment of test accuracy.

Implications for service provision

Owing to a lack of studies addressing the appropriate populations and employing appropriate imaging methods it is unclear whether or not FAF imaging is accurate for the diagnosis and monitoring of retinal conditions in clinical practice.

Any future research into the accuracy of FAF imaging should consider whether FAF imaging is intended to supplement or replace existing imaging modalities. Given that FAF imaging is non-invasive, there might be benefits to patients and the NHS if FAF imaging could replace fluorescein angiography, which is the most frequently used invasive retinal imaging test, although fluorescein angiography would still be needed to assess some aspects of eye disease, for example perfusion. None of the studies included in the current review assessed patients' perceptions of the test procedures or reported whether or not the angiography reference standard was associated with any adverse events. Further evidence would therefore be required to clarify the magnitude of benefits or disadvantages to patients and the NHS of any switch from fluorescein angiography to FAF imaging.

Quality assessment of FAF imaging would be necessary to ensure consistency of diagnostic interpretation. The primary studies included in the systematic review provided no clear information on how this might be achieved. Although intergrader agreement for interpreting FAF images was good in three studies, this is difficult to extrapolate because of methodological limitations of the studies.

Suggested research priorities

- Prospective studies that conform to the paradigm for test accuracy assessments (i.e. which include a clearly specified population, index test, reference standard and diagnostic outcomes) would be helpful to evaluate the diagnostic accuracy of FAF imaging in the inherited retinal dystrophies, early AMD, GA and CSC.
- Prospective studies that conform to the paradigm for test accuracy assessments would be helpful to evaluate the accuracy of FAF imaging in monitoring the progression of retinal conditions and their response to therapy, alongside current best practice, for the inherited retinal dystrophies, early AMD, GA and CSC.
- Future test accuracy studies for FAF imaging should:
    - recruit participants who are representative of those likely to present for retinal screening in the NHS
    - consider carefully whether FAF imaging is appropriate as an ancillary test or as a replacement for an existing test
    - employ all relevant components of currently used reference standards
    - clearly report the clinical information required to interpret FAF images in order to reach diagnostic and/or therapeutic decisions
    - report intergrader and intragrader agreement and other aspects of test acceptability (e.g. patient acceptability, adverse events)
    - and report clearly the duration of imaging and any resources associated with the acquisition, processing, quality assurance and interpretation of FAF images.
- A survey or audit of the current use of FAF imaging in NHS practice would be helpful to clarify current practice and any limitations and research requirements associated with it.

Study registration

This study is registered as PROSPERO CRD42014014997.

Funding

Funding for this study was provided by the Health Technology Assessment programme of the National Institute for Health Research.

Chapter 1 Background

Description of the underlying health problem

Retinal conditions are diseases associated with the retina, that is, the part of the eye that collects light and converts it into electrical signals. Many different conditions can affect the retina, and these vary according to whether they are inherited or acquired, which parts of the retina they involve, whether they are acute or chronic, the extent to which they affect a person's vision, whether they can be treated and at which ages they occur. Retinal conditions also vary according to whether or not they are limited to the retina or affect other parts of the body (the latter are referred to as retinal syndromes). Owing to the diversity of retinal conditions, some of which are very uncommon, it is not possible to describe them all in this report. Since the objective of this technology assessment is to evaluate the accuracy of fundus autofluorescence (FAF) imaging for diagnosing and monitoring retinal conditions, we describe those retinal conditions which, according to the project's clinical advisors, could be amenable to diagnosis and/or monitoring using FAF imaging, or those where FAF imaging may already be in use. To help in understanding the terminology relating to retinal conditions and FAF imaging, we first explain the structures comprising the retina and related parts of the eye.

Overview of the retina and related parts of the eye

The interior surface at the back of the eye, opposite the lens, which can be viewed through the pupil with an ophthalmoscope, includes the retina, and is referred to as the ocular fundus. As well as the retina, the fundus includes the optic disc (where axons exit the eye to form the optic nerve), the macula (a yellow spot which includes the centre of the retina), the fovea (the central part of the macula responsible for sharp central vision) and the posterior pole (the posterior retina as seen with a slit lamp lens, including the macula, optic disc and area nasal to the disc). The left and right halves of the retina (i.e. either side of the fovea) are referred to as 'temporal' (the side towards the temple) or 'nasal' (the side towards the nose). Measurements of the retina are often expressed in degrees of visual angle. One degree of visual angle on the retina is equal to 288 µm, assuming no shrinkage.

When the eye functions well (i.e. without opacity due to cataracts or scars on the cornea, or other visual loss), light entering the eye is focused by the cornea and lens onto the retina. The retina is a highly complex structure comprising several layers and diaphanous membranes (*Figure 1*). Light entering the retina first passes through a layer of neurons before reaching light-sensitive photoreceptor cells (rods and cones), which convert the light energy into electrical signals. The electrical signals are then passed to neurons in the layer above the photoreceptor cells where they are then transferred, via a layer of nerve fibres near the surface of the retina, to the optic nerve, which connects directly with the brain. The photoreceptors are supported towards the outside by a layer of cells called the retinal pigment epithelium. The retinal pigment epithelium reduces light scatter within the eye, provides nourishment and growth factors for the maintenance and regeneration of the photoreceptors, and limits the passage of fluid and harmful substances into the retina. Beneath the retina are a vascular layer called the choroid, and the outside of the eye, known as the sclera. The innermost layer of the choroid is called Bruch's membrane. Blood is supplied to the retina by the central retinal artery, which runs alongside the optic nerve and has four main branches that supply blood vessels in the choroid.

There are two types of photoreceptors, rods and cones. Rods aid vision in low light levels, as well as providing peripheral vision and movement perception; they are found throughout the retina, but the very centre of the retina (1-degree diameter) is rod free with a high cone density. Cones in the macula enable detailed vision of objects directly in front of the viewer and the perception of colour. Cones are also located throughout the retina but their highest density is at the fovea.

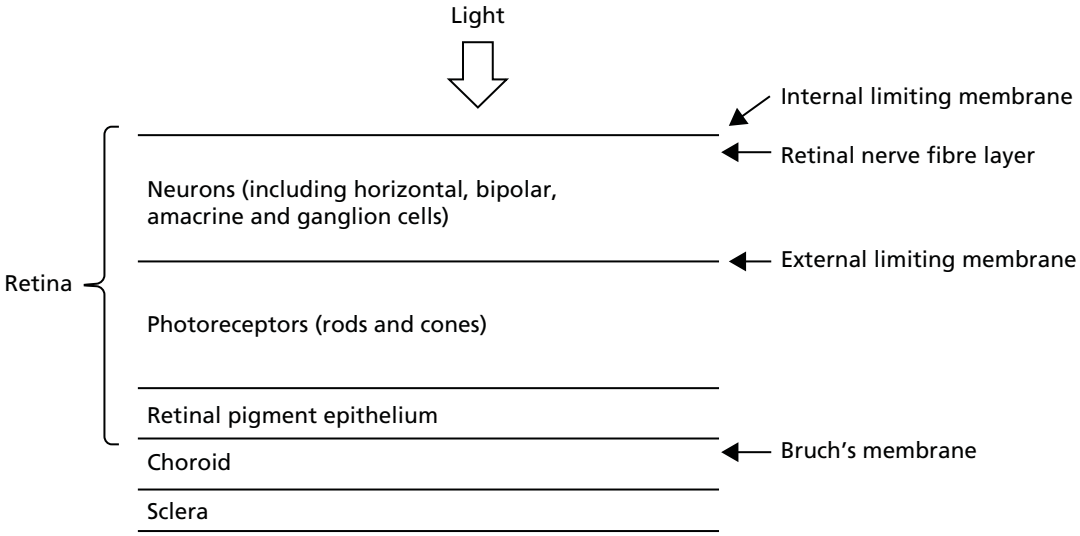

FIGURE 1 Simplified schematic overview of retinal structure (not to scale).

Given the complexity of the retina, and the fact that any component of the retina and its surrounding structures can malfunction or become damaged, a diverse range of retinal conditions can occur.

Retinal conditions included in this review

In this report the term 'retinal condition' (or retinopathy) refers to any acquired or genetically determined disease of the retina. The protocol for the present research focused on the retinal conditions agreed by the project advisory group as being those for which the technology under review, FAF imaging, may be most useful. These are early and late age-related macular degeneration (AMD), geographic atrophy (GA), inherited retinal dystrophies and central serous chorioretinopathy (CSC). However, as explained in *Chapter 3, Inclusion and exclusion criteria*, the scope of the review was subsequently widened to include any retinal conditions (other than those caused by malignancy, major primary ocular conditions or trauma) in which the accuracy of FAF imaging as a diagnostic or monitoring test has been assessed. Where possible, descriptions of retinal conditions in the current report are based on the classification of Lois and Forrester.[1] Below we describe some common retinal conditions and those with significant impacts on patients; due to the wide variety of retinal conditions that exists, this list is not exhaustive.

Prevalence, natural history, symptoms and risk factors of retinal diseases

The estimated UK prevalence of different retinal diseases is summarised below, although this is difficult to compare accurately across the retinal conditions owing to the scarcity of epidemiological data on incidence and prevalence of visual impairment in the UK,[2] and because of differences in how prevalence data have been calculated. As indicated in the sections on specific retinal conditions below, in some cases prevalence rates have been reported without clearly specifying the time periods they refer to. Overall, according to the Royal National Institute of Blind People (RNIB) Eye Health Data Summary 2014[3] (which does not include the less prevalent retinal conditions), based on data for 2010, early AMD is the most prevalent retinal condition in the UK (1,494,000 cases), followed by early diabetic retinopathy (748,000 cases) and late AMD (609,000 cases). Other retinal conditions affect fewer people but, in the case of the inherited retinal dystrophies, symptoms may appear and become debilitating in childhood or young adulthood, unlike in the more prevalent conditions. Where data are available for incidence and prevalence of retinal conditions these are reported in the sections on specific retinal conditions below. For some conditions only incidence or prevalence data are available, not both.

Age-related macular degeneration

Age-related macular degeneration is categorised as either early AMD or late AMD. In early AMD the vision is initially unaffected, but there are risk features for late AMD. Late AMD is classified into two types: wet AMD, which is also known as exudative or neovascular AMD (the term neovascular is used in this report); and dry AMD, which, in advanced cases, is also known as GA. AMD is the commonest cause of visual impairment, and results in progressive, irreversible damage to the macula, the retinal pigment epithelium and retinal photoreceptors.[4] Clinical and phenotypic variations have been identified, and increasing age and smoking are risk factors for the condition.[5] Although AMD usually affects both eyes, it rarely results in complete blindness, as the peripheral vision is not usually affected.

Early age-related macular degeneration

In the early stages of AMD, lipid and basal laminar waste products are deposited under the retinal pigment epithelium[6] and in Bruch's membrane,[7] due to retinal pigment epithelium cells becoming less efficient as people age.[8] These deposits, called 'drusen', are thought to result from incomplete metabolism by retinal pigment epithelial cells.[6] Drusen gradually increase in number and size, although vision is not affected at first. However, central vision becomes less sharp as drusen become larger. Different types of drusen include hard discrete yellow drusen smaller than 50 µm in diameter; tiny whitish basal laminar drusen; soft yellowish drusen with poorly defined margins that often coalesce, and are usually larger than 50 µm; and discrete, calcific crystalline drusen, which look like yellow spots on the retina.[9] Drusen can spontaneously disappear in patients with AMD, often leaving atrophic lesions.[10] With advances in retinal imaging, reticular pseudodrusen have been identified which, unlike 'conventional' drusen, are located within the retinal pigment epithelium.[11,12] The retinal pigment epithelium also undergoes progressive changes in early AMD that appear as patches of hyperpigmentation and hypopigmentation.[7] As noted above, the prevalence of early AMD in the UK has been estimated (based on data from 2010) as 1,494,000 cases.[3]

The sight of people with early AMD may deteriorate gradually over years, and, in some cases, the disease progresses to the more severe GA or neovascular AMD (see *Late, neovascular AMD*), with genetic and environmental risk factors playing a part.[13] About 250,000 older adults in the UK suffer from blindness due to late AMD,[14] and the two main late AMD phenotypes, GA and neovascular AMD, account for two-thirds of registrations for visual impairment or blindness in the UK.[7]

Late, dry age-related macular degeneration (geographic atrophy)

Advanced dry AMD involving atrophy of the retinal pigment epithelium as a result of cell death, is known as GA.[15] GA progresses slowly from early AMD; the evolution from high-risk large drusen to hypopigmentation to death of retinal pigment epithelial cells to legal blindness (Snellen visual acuity < 3/60) can take between 5 and 10 years,[8] and varies considerably between patients.[16] Patients can experience loss of central vision and have difficulty reading small sizes of print, although this may not be noticeable if GA affects only one eye.[7] GA accounts for 12–20% of legal blindness in AMD, but some people retain visual acuity because the atrophy is away from the part of the retina where images are focused, as in foveal-sparing GA.[6] Increasing age, genetic factors and smoking have been reported as the most consistent factors leading to GA.[15] The UK annual incidence rate of GA is 2.4 per 1000 in women and 1.7 per 1000 in men.[17] In the UK, prevalence of dry AMD (based on data from 2010), has been estimated as 194,000 cases.[3] Prevalence rates of GA (based on data for 2007–9) have been reported as 1.3%, 2.6% and 6.7% in people aged over 50 years, over 65 years, and over 80 years, respectively.[17]

Late, neovascular age-related macular degeneration

Neovascular AMD may result in rapid deterioration in vision.[18] Neovascular AMD is characterised by choroidal neovascularisation, which is the development of new blood vessels in the choroid.[7] With the development of new blood vessels, fluid accumulates leading to symptoms of visual distortion. If there is leakage of blood the vision will deteriorate significantly so treatment is needed before permanent damage occurs. As photoreceptors are displaced by fluid, vision becomes distorted and blurred and, without intervention, cell loss, fibrosis and eventual scarring are likely.[7] Patients may suddenly become unable to read, drive and see fine detail, such as facial expressions and features, because of haemorrhaging, fibrosis

or scarring.[7] There are about 26,000 new cases of neovascular AMD per million each year in the UK,[7] and women have slightly higher age-specific prevalence rates than men, although the greater number of older women in the UK results in sex differences in the number of prevalent and incident cases.[17] The UK prevalence of neovascular AMD (based on data from 2010) has been estimated at 415,000 cases.[3] Prevalence rates based on data for Wales from 2005–9 were reported as 1.2% among people aged over 50 years, 2.5% in those aged over 65 years and 6.3% in those aged over 80 years.[17]

Diabetic retinopathy

Diabetic retinopathy is the leading cause of vision loss in people aged under 50 years in the developed world. Its prevalence is increasing,[19] and it is the commonest cause of blindness certification in working age adults.[2] Diabetic retinopathy is a term that describes any pathological features that occur at the retina due to diabetes. This can range from minimal non-sight-threatening vascular features such as microaneurysms, to proliferative retinopathy, which is the growth of delicate new blood vessels that can bleed easily and may result in intraocular haemorrhage with sudden loss of vision.[20,21] Proliferative retinopathy may be transient if the haemorrhage clears, but may have features of advanced disease with an associated retinal detachment. Other types of diabetic retinopathy include fluid collecting at the macula (diabetic macular oedema) or loss of blood vessels at the macula, known as macular ischaemia, which can be associated with severe central visual loss depending on the extent of the loss of vascular perfusion.[22]

The most common symptom of diabetic macular oedema is blurred vision. Other symptoms include metamorphopsia (distortion of the visual image); floaters (moving spots seen in the field of view); loss of contrast sensitivity; photosensitivity (intolerance to light); changes in colour vision; scotomas (where part of the visual field is missing); vision becoming blurred, hazy or fluctuating, or with the appearance of black lines or dots; periods of temporary 'blackness' caused by retinal haemorrhage; poor peripheral vision; and poor depth perception.[23] People with diabetic macular oedema may also be affected by a difference in sight between their two eyes.[22] Laser treatment of macular disease or proliferative retinopathy can also lead to visual symptoms including field loss and photosensitivity.

Estimates of UK prevalence of diabetic retinopathy vary owing to the range of different sources of data. Based on data from 2010, there is an estimated prevalence of 748,000 cases of early diabetic retinopathy in the UK, 85,000 cases of more advanced retinopathy and 188,000 cases of diabetic maculopathy.[3] In a study of people with diabetes in England (which also estimated prevalence data for 2010), 7.12% had diabetic macular oedema in one or both eyes, of which 39% had clinically significant diabetic macular oedema.[24]

Central serous chorioretinopathy

Central serous chorioretinopathy is the fourth most common retinopathy after AMD, diabetic retinopathy and retinal vein occlusion.[25] It is characterised by fluid accumulating as a result of dysfunction of retinal pigment epithelial cells and/or the choroid. Usually, if the patient experiences symptoms, the fluid will have accumulated under the central macula.[26] Spontaneous visual recovery can occur within 2 or 3 months in the acute type,[25] but chronic CSC, which is more common in older people, can lead to persistent or episodic blurred vision. In some cases of chronic CSC, disease progression is more severe, leading to retinal pigment epithelial atrophy,[27] which may result in permanent visual loss.[25] There is some evidence that risk factors for CSC include smoking, hypertension or sympathetic responses (nervous system activation in response to stressors),[27] steroid medications[28] or genetic susceptibility. Symptoms of the condition include image size disparity between the eyes, blurred central vision, altered colour vision and, in chronic disease with atrophic change, a loss of visual acuity.[25]

Men are more often affected by CSC than women, in a ratio of up to 8 : 1.[26] Although predominantly an adult disease, some cases of CSC have been reported in children.[26] People aged over 50 years are more likely to have the condition in both eyes, may develop choroidal neovascularisation[25] and may have coincidental polypoidal choroidopathy. CSC recurs in about one-third of patients within around 1 year.[25] The peak incidence is around 40–45 years in men, but later in women, at about 51 years.[26] Reliable incidence and prevalence data are not available for the UK. According to a study in the USA (based on data for 1980–2002), the incidence of CSC is about 9.9 per 100,000 in men and 1.7 per 100,000 in women.[25]

Inherited retinal dystrophies

Inherited retinal dystrophies are a broad group of genetically determined disorders that affect the retina and lead to progressive visual loss. These disorders can be generally classified according to whether they affect primarily the centre of the vision, the periphery or both. Further classification depends on whether rods or cones are primarily affected, whether or not both central and peripheral systems are involved and which is more affected (referred to as rod–cone or cone–rod).[29] Macular dystrophies may only involve central vision without affecting the visual fields, and the rods and cones may not be affected. However, progression may occur so that, for example, a disorder that was classified as macular dystrophy may, in time, develop cone and rod dysfunction. Terms used to describe these conditions depend on the appearance of the retina, which cells are involved, and (more recently) if the gene mutation(s) is/are known. Retinal dystrophies include, among others, retinitis pigmentosa, macular dystrophy, Stargardt disease (which primarily develops in childhood and adolescence) and Best disease.

The age of onset of retinal dystrophies varies, with early and late onset forms having different inheritance patterns, and mutations in over 200 different genes are known to be involved in different types, including syndromic retinal dystrophies.[30] Symptoms and disease progression also vary within each named condition. Symptoms may be apparent from birth or may appear at any age, depending on the type and the specific genetic variant inherited. The degree of central visual loss, peripheral field loss and difficulty with seeing in the dark depends on how well each group of photoreceptor cells is working, and the speed of sight loss varies with different genetic forms and between individuals.[31] In cases of severe rod–cone dystrophy with early onset, macular involvement may lead to early decline of visual acuity, and these cases may be more challenging to diagnose.[29]

Although some inherited retinal dystrophies affect few people, overall, these disorders are stated as being a cause in 15.8% of certified blindness registrations and 10% of partial sight registrations in England and Wales, and are considered to be a significant burden in the working age population.[2] The annual incidence of hereditary retinal disorders in the UK has been estimated at around 1500 cases, and about 100 children and 480 adults of working age in the UK are registered as blind or partially sighted as a result of these conditions.[2] A study published in 2012[32] estimated that (based on data from 2006 to 2008) the annual UK incidence of childhood-onset retinal dystrophies, is 1.4 in 100,000 children (aged 0–15 years) and the cumulative incidence by age 16 years is 22.3 in 100,000 children.

Cystoid macular oedema

Macular oedema is the accumulation of fluid within the retina at the macular area, secondary to various retinal conditions. Depending on its cause, acute oedema may resolve spontaneously.[33] Cystoid macular oedema is the result of chronic oedema that persists for ≥ 4 months, leading to the formation of cystic honeycomb-like spaces in the retina,[34] and can occur as a consequence of retinal dystrophies,[35] inflammatory diseases (uveitis, scleritis, birdshot chorioretinopathy, toxoplasmosis),[36] retinal vascular disease (retinal vein occlusions, idiopathic retinal telangiectasia, radiation retinopathy),[34] diabetic retinopathy,[34] cataract or other eye surgery, injury to the eye, choroidal tumours, or may be drug induced, for example with prostaglandins such as latanoprost.[37] As with other macular conditions, the main symptoms of cystoid macular oedema are blurred or decreased central vision, but peripheral vision is unaffected. Estimates of the incidence and prevalence of cystoid macular oedema vary considerably depending on the cause of the oedema. For example, the incidence of clinically significant cystoid macular oedema caused by uncomplicated cataract surgery has been estimated across studies as 0.6–2.6%,[38] whereas the prevalence of cystoid macular oedema in patients with retinitis pigmentosa may be as high as 32–49% (unilateral) or 18–44% (bilateral).[39,40]

Impact of retinal conditions

Significant distress results from developing visual loss,[41] particularly when the loss is marked and/or of rapid onset. Depending on the condition, patients can experience a wide range of symptoms, as described above. Such symptoms can have a profound impact, for example, the loss of central vision associated with AMD affects patients' ability to perform normal daily activities such as reading, writing and recognising faces.[18] People with diabetic retinopathy may experience a visual difference between their two eyes.[22]

Visual loss adversely affects people's ability to drive, which has a serious negative impact on work, social life, relationships, responsibilities and independence.[22,42] Visual loss also adversely affects self-care, increases the risk of falls, hip fractures and early admission to residential care, and adversely affects quality of life.[18] The psychological impact of changes caused by vision loss include alterations to the self-concept, life goals and social functioning.[43] Having to adapt to vision loss may result in emotional distress, which can lead to depression, anxiety and sleep problems.[18,43]

Diagnosis and monitoring of retinal conditions may involve invasive procedures such as fluorescein or indocyanine green angiography (ICGA), which involve intravenous injections of dye. People with retinal conditions may require frequent treatments and regular monitoring of their response to treatment and disease progression, which may be uncomfortable and can cause anxiety and apprehension.[18] With the ageing population and recent treatment advances, demand for early identification of people who are at greatest risk of progressive disease is also increasing.[44] More accurate and less-invasive diagnostic procedures may therefore be helpful if they can improve the efficiency of diagnosis and/or monitoring of retinal conditions.

Measurement of disease: diagnostic features

Imaging techniques capture morphological patterns (appearance of structural features) at the back of the eye, as well as dynamic features such as transit of dye in blood vessels. These features are seen more or less clearly with different imaging modalities, for example, pooling, leakage or staining can be seen with fluorescein angiography. Grading systems have been proposed to classify different conditions, using different imaging modalities, although there is not always universal consensus about the most appropriate classification for each condition. For example, the Wisconsin age-related Maculopathy system consists of three sections: drusen, other lesions and other abnormalities, each of which includes subgroups of characteristics.[6]

A classification by the International Fundus Autofluorescence Classification group describes patterns of fluorescence in early AMD and GA using FAF imaging.[45,46] With advances in imaging, reticular pseudodrusen have been identified, which unlike conventional drusen are located within the retinal pigment epithelium,[11] and these are characteristic of AMD.[47] Determining the type and number of drusen present allows clinicians to better inform patients of their risk of developing AMD.

Geographic atrophy is defined as any sharply delineated round or oval area of hypopigmentation (paleness), or apparent absence of the retinal pigment epithelium and overlying photoreceptors, in which choroidal vessels are more visible than in surrounding areas, and the oval area is > 175 µm in diameter.[15]

Diabetic macular oedema is characterised by macular thickening (focal, multifocal or diffuse) or hard exudates within 500 µm of the centre of the macula. Cystoid macular oedema is also characterised by retinal thickening and the development of honeycomb-like spaces in the retina on optical coherence tomography evaluation.[34]

In inherited retinal dystrophies, clinical signs may indicate the type of dystrophy. For example, in retinitis pigmentosa, pigment changes (known as bone spicules because they look like bone under the

microscope), pallor of the optic disc and narrowing of retinal blood vessels may be seen. Stargardt disease is characterised by white/yellow flecks at the back of the eye between the optic disc and the macula, seen by fundoscopy, colour imaging or autofluorescence, and can extend to cover a larger area of the retina.[48] In most patients some of the flecks will atrophy, leaving lesions with a beaten metal appearance on an electroretinogram.[48] Patients with cone–rod disorders may have pigment deposits in the retina, mainly in the macular region,[29] or little or no pigment change but a 'bull's-eye maculopathy' on fundus examination.[49] As diagnostic features of inherited retinal dystrophies overlap, for example Stargardt disease can be mistaken for pattern dystrophy and vice versa, genetic testing plays a helpful and necessary part in clarifying which disease is present.

Description of the technology under assessment

Fundus autofluorescence in relation to retinal health

The fundus of the human eye has a 'natural' or 'background' level of fluorescence, which is referred to as autofluorescence. This is caused by the presence of molecules with fluorescent properties (i.e. molecules that, when exposed to light of an appropriate wavelength, absorb the incident electromagnetic energy and re-emit this as light at wavelengths longer than those of the initial source). The fluorescent molecules, known as fluorophores, are potentially of clinical value in detecting age- or disease-related processes, since their density and distribution alters with ageing of the eye and with certain pathological conditions. Notably, lipofuscin is a fluorescent pigment that accumulates in the retinal pigment epithelium as a by-product of cell metabolism and can lead to the development of drusen. Lipofuscin deposition normally increases with age but its accumulation may also reflect cell dysfunction or metabolic abnormalities in the retinal pigment epithelium. Excessive lipofuscin deposition in the retinal pigment epithelium is considered pathologic and is associated with visual loss.[50] FAF imaging techniques have potential value as diagnostic tools, since the presence and distribution of fluorophores may correlate with disease activity and may provide an early indication of future disease development, progression or response to treatment. FAF imaging is also potentially valuable in detecting areas of reduced or absent autofluorescence (hypoautofluorescence), which can arise either through atrophy of retinal structures or blocking of FAF reflectance (e.g. by blood vessels).

Fundus autofluorescence imaging techniques

The intensity of light emitted during FAF is relatively weak (about two orders of magnitude lower than the background of a fluorescein angiogram at peak dye transit) and so specialist imaging techniques are required to enable FAF to be detected and mapped. FAF has been an area of interest in ophthalmic research for more than 40 years but it has only recently become clinically relevant, as a result of technological advances.[50] As outlined below, there are three main methods for assessing FAF: using a fundus camera, fundus spectrophotometry or confocal scanning laser ophthalmoscopy (cSLO).

The fundus camera uses a single flash to image the entire retinal area instantaneously. The acquired autofluorescence image is derived from all tissues in the light beam with fluorescent properties; light scattered anterior and posterior to the plane of interest can greatly influence the detected signal.

Fundus spectrophotometry was developed to measure FAF from small retinal areas (2 degrees of visual angular diameter). It incorporates an image intensifier diode array as a detector and beam separation in the pupil to minimise the contribution of autofluorescence from the crystalline lens.[51]

In cSLO a focused low-power laser beam is swept across the fundus in a raster pattern (horizontal and vertical scanning across a grid) to provide the excitatory light source for fluorophores.[52] Different excitation wavelengths can be generated, depending on the type of laser used, including 488 nm (blue light) with a solid-state laser and 514 nm (green light) with an argon-ion laser. For near-infrared fundus autofluorescence (NIR-FAF), the excitation wavelength is 790 nm. The confocal nature of the optics reduces the detection of autofluorescence from structures anterior to the retina, such as the lens and cornea. Unlike fundus

spectrophotometry, cSLO allows imaging of FAF over larger retinal areas [e.g. 55 degrees in Heidelberg Retina Angiograph-based systems (Heidelberg Engineering, Heidelberg, Germany)]. To reduce background noise and enhance image contrast, the mean image of several FAF images is obtained (usually based on up to 20 frames, after adjustments to correct for eye movement). In order to block the reflected light but permit autofluorescence light to pass, cSLO have barrier filters of 500 nm, 525 nm and 800 nm for excitation wavelengths of 488 nm, 514 nm and 790 nm, respectively. As well as the excitation and barrier filter wavelengths, image contrast and brightness settings also differ among cSLO devices and these differences must be taken into account when comparing the results of FAF imaging obtained from different cSLO devices.[16]

Confocal scanning laser ophthalmoscopy is the most sensitive imaging approach for identifying autofluorescence that arises specifically from the fundus (i.e. minimising the detection of autofluorescence arising from other parts of the eye such as the lens).[16] According to clinical experts advising the review, cSLO is the current standard method employed for obtaining FAF images of retinal conditions, and is therefore the only type of scanning laser ophthalmoscopy permitted as an index test for assessing FAF in the current review.

Fundus autofluorescence for diagnosis and monitoring of disease

The quantitative accuracy of FAF imaging for the diagnosis and/or monitoring of retinal conditions (i.e. its sensitivity and specificity) is unclear. However, FAF imaging is considered helpful in a number of conditions to help establish a diagnosis and monitor treatment without the need for angiography,[1] is relatively easy to accomplish and requires little time.[53] Studies have suggested the potential diagnostic value of FAF imaging as a more sensitive marker of retinal pathology than existing examinations alone, for example, in GA,[54] choroidal neovascularisation development in AMD,[55] retinal pigment epithelium alterations,[56] Best disease,[57] cystoid macular oedema[58] and CSC.[59] Clinical advisors to the current review also suggested that FAF is useful for diagnosing any type of inherited retinal dystrophy, and has the potential to show pathologic features earlier than on fundoscopy. FAF imaging also appears promising for monitoring changes in a number of retinal conditions, either natural progression or responses to therapy. FAF imaging has been used, for example, for monitoring changes in GA;[60–66] retinal pigment epithelium tear or loss;[67–69] retinitis pigmentosa;[70,71] Stargardt disease,[72] CSC,[73] diabetic macular oedema[74] and retinal vasculitis.[75] Increasingly, FAF has been specified as an end point in clinical research studies of therapies for retinal conditions, for example, with antivascular endothelial growth factor drugs,[68,76–78] sirolimus,[65] lampalizumab,[66] finasteride,[73] photodynamic therapy[79] and vitrectomy.[80]

Reference standards

'Reference standard' refers to the current gold standard or best available method for accurately identifying a given retinal condition. A reference standard may consist of more than one retinal imaging test since different imaging modalities can provide different types of information which, when interpreted together, improve diagnosis. According to the paradigm for assessing test accuracy, the reference standard (against which any new tests will be compared) should have high (ideally 100%) sensitivity and high (ideally 100%) specificity for identifying the retinal condition, that is, the method should minimise false-positive and false-negative results.[81] The extent to which existing methods used in diagnosing retinal conditions fulfil these strict requirements is not always clear and there is not always agreement among clinicians on which method is the 'best'. In early AMD, optical coherence tomography and fluorescein angiography are needed to distinguish between wet and dry AMD,[82] but ICGA may be also used, for example if there is doubt about the presence of choroidal polyps or chorioretinal anastomoses.

Clinical advisors to the current review suggested that spectral-domain optical coherence tomography would be a standard approach for identification of reticular pseudodrusen in AMD, possibly also with fluorescein angiography and/or colour fundus photography. GA diagnosis is confirmed by clinical examination using a high-definition fundus lens for stereo biomicroscopy, as well as being noted on

fluorescein angiography.[7] As noted above, FAF imaging and spectral-domain optical coherence tomography have made it easier to diagnose GA, particularly early signs of GA, as these imaging modalities can reveal areas of GA that may not be clinically visible on biomicroscopy.[7] The gold standard for diagnosing cystoid macular oedema has been specified as fluorescein angiography,[38,83] although in practice optical coherence tomography is also important[34,38] as diagnosis involves assessment of macular thickness as well as assessment of perfusion of the retinal vascular epithelium. Colour fundus photography, and more recently optical coherence tomography, are the reference standards for assessing diabetic macular oedema.[84] Fluorescein angiography may be used to identify leaking microaneurysms or capillaries and areas of macular ischaemia, as well as checking the rest of the retina for ischaemia or neovascularisation. Indocyanine green angiography is a standard approach for detecting choroidal abnormalities in CSC, and enhanced depth optical coherence tomography can provide three-dimensional information.[85] Diagnosis of retinitis pigmentosa involves visual field testing, electroretinography to measure the functional status of photoreceptors and stereo fundus biomicroscopy. Diagnosis of Stargardt disease may be based on visual acuity, fundus examination, electroretinography and fluorescein angiography, although optical coherence tomography and microperimetry may also be useful.[86]

Current service provision

Although FAF imaging may be used on an ad-hoc basis in the NHS to support the diagnosis and monitoring of a range of retinal conditions, the extent to which it is used in practice is unclear. Clinical experts advising the current review suggested that the use of FAF imaging depends on the eye unit, its field of expertise and specialism. Although the role of FAF imaging is still evolving, the method is already widely used within the NHS for the assessment of inherited retinal dystrophies, such as retinitis pigmentosa and Stargardt disease, as high-resolution imaging of the distribution and levels of FAF correlate with pathogenesis in these conditions (e.g. indicating areas where the retina is dying). This is, however, in centres where there is provision of an inherited retinal dystrophy service and often a strong associated research unit. The extent to which FAF imaging is used for diagnosing and/or monitoring GA and CSC is unclear, but clinical experts have suggested that FAF imaging would mainly be used in specialised retinal clinics such as those seen in teaching hospitals. FAF imaging is unlikely to be used in isolation and would commonly be conducted together with other tests such as (depending on the retinal condition) optical coherence tomography, fundus photography, fluorescein angiography or indocyanine green angiography. FAF imaging would complement these tests rather than replace them, since the imaging modalities provide different information to help clinicians reach a diagnostic or therapeutic decision. An exception could be for the inherited retinal dystrophies where FAF imaging has potential to replace fluorescein angiography, for example, as the main test for diagnosing Stargardt disease (FAF could be an alternative indicator of disease by demonstrating flecks and atrophy, where previously fluorescein angiography was performed with the aim of confirming Stargardt disease if a dark choroid was identified). The clinical experts suggested that, for the diagnosis or monitoring of neovascular AMD, choroidal neovascularisation and macular oedemas (cystoid and diabetic), FAF imaging would provide little or no added value over optical coherence tomography and fluorescein angiography. This is because the accumulation and/or leakage of fluid in these conditions could mask the FAF signal, which might make the assessment of the distribution and intensity of autofluorescence unreliable, and also because dye leakage, as assessed using angiography, is necessary for confirming choroidal neovascularisation and macular oedema.

According to the Royal College of Ophthalmologists' guidelines,[7] FAF imaging along with spectral domain optical coherence tomography has made it easier to diagnose GA in AMD, but its role in diabetic retinopathy has yet to be fully elucidated.[20] Despite the potential value and existing use of FAF imaging in the inherited retinal dystrophies, no guidelines are currently available concerning the use of FAF imaging in diagnosing these retinal conditions.

Chapter 2 Definition of the decision problem

Decision problem

Fundus autofluorescence imaging appears to be a promising procedure for the diagnosis and/or monitoring of certain diseases of the retina.[50] Although FAF imaging is already employed (to an unclear extent) in the NHS, its accuracy for the diagnosis and monitoring of the different retinal conditions is uncertain. A systematic evaluation of both the quantity and the quality of the available evidence on the diagnostic and monitoring accuracy of FAF imaging is needed to inform best practice for retinal imaging in the NHS and to inform future research.

Population

The relevant population is patients of any age who are suspected to have, or have previously been diagnosed with, any retinal conditions, excluding those resulting from malignancy, other ocular diseases (such as glaucoma) or trauma. Note: this population is wider than that specified in the review protocol, as it is not limited to specific retinal conditions (see *Chapter 3, Inclusion and exclusion criteria*).

Index test

The relevant index tests are any FAF imaging approaches performed to assist diagnosis and/or monitoring of retinal conditions, using cSLO. As noted above, in some retinal conditions such as GA and Stargardt disease, FAF imaging may already contribute information to making the diagnosis. The use of FAF imaging as an index test has to be considered carefully because interpretation of its diagnostic or monitoring accuracy may be influenced by whether or not it is already part of the reference standard and by whether results of FAF imaging would normally be interpreted in isolation or in conjunction with other clinical information (e.g. results of other imaging tests). These issues are an important part of the critical appraisal of the available evidence and are considered in detail in this review when interpreting results (see *Chapter 4*).

Reference standard

To account for the wide range of retinal conditions eligible for inclusion in the review and differences in how reference standards have been defined and employed in various studies, fundus imaging performed using any clinically relevant method (e.g. fundus photography, fundus fluorescein angiography, indocyanine green angiography, optical coherence tomography or any combination of relevant tests) is eligible for inclusion in the current review.

Outcomes

The key outcome measures include: sensitivity and specificity; positive and negative likelihood ratios; positive and negative predictive values; and diagnostic odds ratios. The working definitions of each of these outcomes are:[87]

- Sensitivity: true identification of the people with the condition of interest. It is also known as the true-positive rate. A high sensitivity implies that a negative result rules out a condition.
- Specificity: also known as the true-negative rate, it indicates the true identification of people without the condition. A test with high specificity implies that a positive result confirms the condition.
- Likelihood ratios: a positive likelihood ratio is the ratio of the true-positive rate to the false-positive rate, which is expressed as: sensitivity/(100 – specificity) whereas a negative likelihood ratio is the ratio of the false-negative rate to the true-negative rate, expressed as: (100 – sensitivity)/specificity. The positive likelihood ratio describes how many times more likely positive index test results are in the group with the retinal condition compared with those without the condition, and should be > 1 for the test to be informative. The negative likelihood ratio describes how many times more likely negative index test results are in the group with the retinal condition compared with those without the condition, and should be < 1 for the test to be informative.
- Positive and negative predictive values: positive predictive value is the probability of the condition of interest among people with a positive test result. Negative predictive value is the probability of not having the condition among people with a negative test result.
- Diagnostic odds ratio: this summarises the diagnostic accuracy of the index test in a single number that describes how many times higher the odds are of obtaining a positive test result in a patient with the retinal condition than in one without the condition.

Overall aims and objectives of the assessment

The aim of this project was to assess the accuracy of FAF imaging using cSLO for the diagnosis and monitoring of retinal conditions. Specific research objectives were:

- for each retinal condition, to determine the diagnostic and monitoring accuracy of FAF imaging using cSLO, including monitoring of disease management
- to identify future research needs and develop research recommendations.

Chapter 3 Methods for reviewing test performance

A review of the evidence for test accuracy was undertaken systematically and is reported systematically following the general principles outlined in the Centre for Reviews and Dissemination guidance,[88] the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy[87,89] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement,[90] taking into consideration specific aspects of methodology that are relevant to the synthesis of evidence of test accuracy.

The project was informed by an advisory group of three independent clinical experts (see *Acknowledgements*). Two of the experts were experienced ophthalmologists and the third represented RNIB, which is a national charity supporting people with sight problems.

Identification of studies

A comprehensive search strategy (see *Appendix 1*) was developed, tested and refined by an experienced information scientist. The search strategy aimed to identify studies on the diagnosis and/or monitoring of relevant retinal conditions based on the prespecified inclusion and exclusion criteria (see *Inclusion and exclusion criteria*). The search strategy comprised the following main elements:

- searching electronic databases
- searching internet pages of relevant organisations, meetings and trial registries
- scrutiny of bibliographies of retrieved papers and relevant systematic reviews.

The following electronic databases were searched: MEDLINE (via Ovid); MEDLINE In-Process & Other Non-Indexed Citations; EMBASE; The Cochrane Library; Web of Science; Database of Abstracts of Reviews of Effectiveness (via the Centre for Reviews and Dissemination); Health Technology Assessment database; Medion database of diagnostic accuracy studies.

Internet pages of the following organisations were searched: American Academy of Ophthalmology, Association for Research in Vision and Ophthalmology; Cochrane Eyes and Vision Group; European Association for Vision and Eye Research; and Royal College of Ophthalmologists.

Searches for grey literature and research in progress included: the UK Clinical Research Network Portfolio Database; World Health Organization International Clinical Trials Registry Platform; Current Controlled Trials; Clinical Trials.gov; and the National Institute for Health Research Clinical Research Network Portfolio.

All databases were searched from 1990 (approximately 10 years prior to the likely publication of the earliest relevant evidence) to November 2014 and searches were limited to the English language.

Inclusion and exclusion criteria

The eligibility criteria for the systematic review are as specified in the review protocol,[91] with two exceptions. First, the population has been widened to include patients with any retinal condition, but excluding those resulting from malignancy, major ocular conditions (e.g. glaucoma) or trauma. This change was made because the pilot screening process identified potentially relevant studies in which FAF imaging had been conducted on retinal conditions not specified in the protocol but did not appear to identify many studies on the prespecified conditions. Widening of the population inclusion criterion was considered acceptable on the basis that it was deemed unlikely to affect resource requirements for the review.

To avoid any inconsistency and bias, the updated population eligibility criterion was applied to all of the bibliographic records screened. Second, the protocol specified that, if appropriate, prospective studies would be prioritised and retrospective studies would be included only if no prospective studies were available for a given retinal condition. However, this criterion was amended so that both prospective and retrospective studies would be included, owing to the limited number of available relevant studies. The eligibility criteria employed were therefore as follows:

- Population: patients of any age who were suspected to have, or have previously been diagnosed with, any retinal conditions, excluding those resulting from malignancy, other ocular diseases (such as glaucoma) or trauma.
- Index test: FAF imaging performed using cSLO.
- Reference standard: fundus imaging performed using any clinically relevant method (e.g. fundus photography, fundus fluorescein angiography, indocyanine green angiography, optical coherence tomography, or any combination of relevant tests).
- Primary outcomes: sensitivity and/or specificity (including any data from which these could be calculated) for the diagnosis or monitoring of retinal conditions.
- Secondary outcomes (applicable only if primary outcomes were also reported): inter- and intraobserver agreement, adverse events, test acceptability to patients and clinicians, and test interpretability.
- Study designs: any prospective or retrospective study design, provided that the index test(s) and reference standard were compared in the same patient group.
- Studies were excluded if they had small sample sizes, that is, fewer than 10 study eyes. Based on published tables of sample sizes required to achieve a given level of diagnostic outcome precision,[92] we considered a minimum sample size of 10 eyes per study appropriate to exclude highly imprecise evidence.

Study selection

Studies were selected for inclusion through a two-stage process using predefined and explicit criteria (as specified in *Inclusion and exclusion criteria*). First, two reviewers independently screened all titles and abstracts identified in the searches to identify bibliographic records that met the inclusion criteria, using a standard pilot-tested study selection worksheet (see *Appendix 2*). Second, full-text articles were retrieved for those bibliographic records judged to be relevant or unclear at the title and abstract screening step. If a study was reported in more than one article, all articles relating to the study were grouped together for assessment. One reviewer assessed eligibility of each study using the study selection worksheet and a second reviewer checked the decision. At each step of the selection process, any disagreements were resolved by discussion between the two reviewers or, if necessary, by involving a third reviewer.

Data extraction

Data extraction was undertaken by one reviewer and checked by a second reviewer using a predesigned and piloted data extraction form to minimise errors. Any disagreements between reviewers were resolved by consensus or, if necessary, arbitration by a third reviewer. Where sensitivity and specificity were reported in the primary studies with confidence intervals these were extracted and checked and, if necessary and possible, missing values were calculated. Positive and negative likelihood ratios, positive and negative predictive values and the diagnostic odds ratio were also checked and, if necessary, calculated where possible.

Critical appraisal

The methodological rigour of studies reporting diagnostic accuracy was assessed using the Cochrane adaptation[81] of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool[93] (which focuses on methodological rigour rather than quality of reporting) (see *Appendix 3*). For each of the included studies, judgements on study rigour were made by one reviewer using the QUADAS criteria and were checked by a second reviewer. Any disagreements between reviewers were resolved by consensus or, if necessary, arbitration by a third reviewer. The QUADAS tool[93] asks 11 questions about the characteristics of the primary studies. These questions aim to identify potential threats to the validity of the study findings, and (in our classification) reflect 10 different types of bias that can be present in studies of test accuracy. The way these are interpreted in the current report is summarised in *Table 1*.

The QUADAS question on 'patient spectrum' takes into consideration two elements: whether or not the population characteristics are likely to be representative of those found in actual clinical practice; and whether the primary study design is retrospective or prospective. In this sense the question combines elements of bias and applicability. The reason is that empirical evidence suggests that retrospectively conducted studies are at high risk of bias and as such this limits interpretation of the population relevance.[94]

In addition to the modified QUADAS criteria, if necessary, a distinction was made between studies that included one or both eyes per individual so as to avoid any unit-of-analysis issues (e.g. using a subgroup analysis approach).[95]

TABLE 1 Types of bias possible in studies of the accuracy of FAF imaging

QUADAS question	Type of bias	Explanation
1	Spectrum bias	The study population is not representative of those who will receive the index test (FAF imaging) in clinical practice
2	Verification bias	The reference standard does not accurately diagnose the target retinal condition
3	Disease progression bias	The time interval between index (FAF imaging) and reference standard tests is long enough that the two tests may not have measured the same retinal disease state
4, 5	Differential verification bias	Diagnosis is inaccurate because not all patients receive the same reference standard test
6	Incorporation bias	The index test (FAF imaging) is not independent of the reference standard (e.g. may be one of several tests used as the reference standard)
7	Diagnostic review bias	The index (FAF imaging) test result influences interpretation of the reference standard result
8	Test review bias	The reference standard result influences interpretation of the reference index test (FAF imaging) result
9	Clinical review bias	The information used when interpreting the index test (FAF imaging) does not reflect that likely to be available in clinical practice
10	Image classification bias	Incorrect inclusion or exclusion from the analysis of index test (FAF imaging) images classified as intermediate or of unclear quality may systematically influence sensitivity or specificity
11	Attrition bias	Exclusion of patients or eyes from analysis may systematically influence sensitivity or specificity if the reason for exclusion is linked to test performance, or if criteria for permitting exclusions differ between imaging tests, especially if the magnitude of attrition is unbalanced across the test methods

Method of data synthesis

Studies were synthesised through a structured narrative review with tabulation of results of included studies. Heterogeneity among studies and analyses of relevant subgroups was explored and presented using paired sensitivity and specificity forest plots. Paired forest plots differ from standard forest plots, which provide a measure of effect on the *x*-axis; one of the paired plots provides a measure of sensitivity on its *x*-axis and the other plot a measure of specificity on its *x*-axis. As such, visualising both plots together can illustrate how both sensitivity and specificity vary among a group of displayed studies. The *x*-axis in both cases ranges from 0 to 1, and in the ideal test both sensitivity and specificity would be 1. The analysis and synthesis followed good practice approaches as recommended by the Centre for Reviews and Dissemination (chapter 2: systematic reviews of clinical tests)[88] and the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy.[87,89]

We planned (subject to the availability and suitability of the primary data) to conduct one or more meta-analyses of data on test sensitivity and specificity, in order to improve the precision of any estimates of the accuracy of FAF imaging for diagnosing or monitoring retinal conditions. The appropriateness of meta-analysis was determined by critical appraisal of the primary studies during the quality assessment step (see *Critical appraisal*). To account for correlation between sensitivity and specificity, and their dependence on the prevalence of retinal conditions, the planned pooling of sensitivity and specificity outcomes was based on appropriate hierarchical random-effects models [using statistical software such as Winbugs (MRC Biostatistics Unit, Cambridge, UK) or SAS (SAS Institute Inc., Cary, NC, USA)].

Chapter 4 Assessment of diagnostic and monitoring studies

This chapter presents the quantity of research available, including the number of studies, their designs, participant characteristics and the characteristics of the index tests and reference standards that they compared (see *Quantity of research available*). Critical appraisal of the included studies is then reported, including their risks of bias (see *Quality of research available*) before the assessment of diagnostic accuracy is presented (see *Assessment of test accuracy*), which takes into consideration the available evidence on diagnostic outcomes as well as any threats to validity highlighted in the preceding sections.

Quantity of research available

The selection of evidence for the systematic review is summarised in *Figure 2*. Searches yielded 2240 unique bibliographic records, the majority of which were excluded on inspection of the title and/or abstract because the record did not meet one or more of the specified criteria listed in the study selection worksheet (see *Appendix 2*). Full-text versions were obtained for 206 bibliographic records for further scrutiny where the title and abstract appeared to meet the inclusion criteria, or insufficient information was available to make a judgement on eligibility. Scrutiny of these 206 full-text articles revealed that the majority (198) were not relevant, primarily because they did not report (or provide sufficient data to enable us to calculate) sensitivity and specificity of FAF imaging. Reasons for excluding each of these 198 full-text articles are listed in *Appendix 4*. The remaining eight full-text articles (all peer-reviewed journal papers) met all of the specified eligibility criteria and are included in the current systematic review.

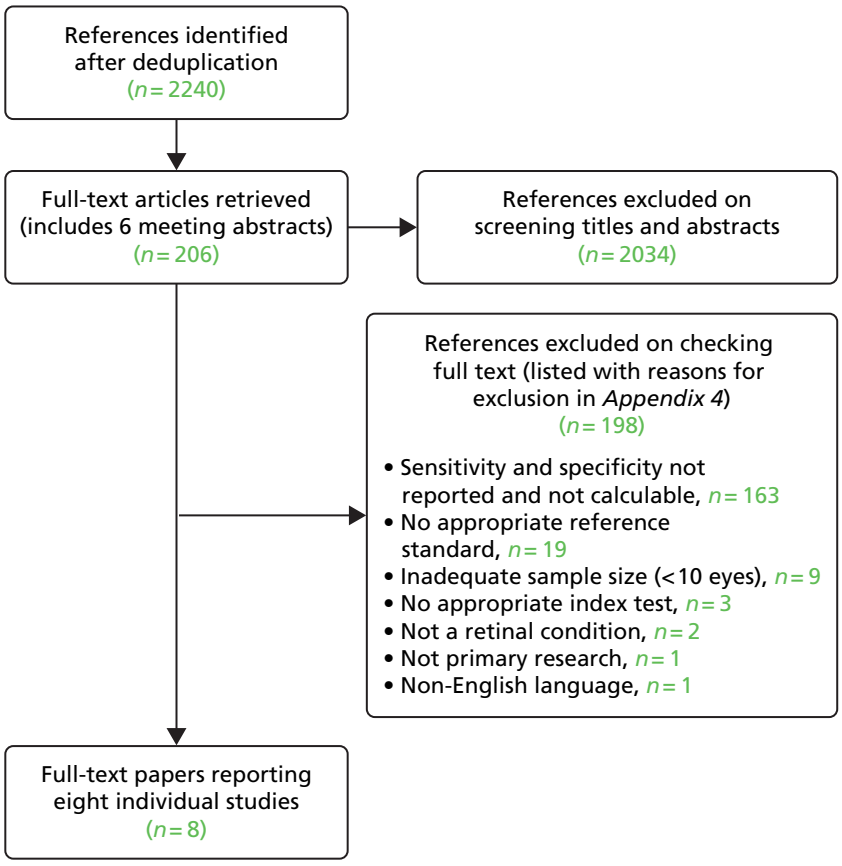

FIGURE 2 Flow chart for the identification of studies.

Number and type of studies included

The eight included research papers describe eight unique primary studies.[83,84,96–101] Full details of these studies, including our assessment of their methodological rigour, are given in the data extraction forms (see *Appendix 5*).

Characteristics of the included studies

Key characteristics of the included studies are summarised in *Table 2*.

TABLE 2 Study designs

Retinal condition	Study	Patient recruitment (for study inclusion criteria see *Appendix 2*)	Prospective research	Consecutive selection of patients (or images)	Diagnosis and/or monitoring	Interobserver agreement reported
AMD: choroidal neovascularisation	Cachulo *et al.*[99]	Patients were actively recruited from one centre	Yes	Not reported	Diagnosis only	No
AMD: reticular pseudodrusen	Hogg *et al.*[96]	Patients were actively recruited from three centres in three countries by invitation	Yes	Not reported	Diagnosis only	Yes, in one out of three study centres only
	Smith *et al.*[101]	FAF and CFP photograph pairs were selected from two databases in different countries	No	Not reported	Diagnosis only	No
	Ueda-Arakawa *et al.*[97]	A series of patients with AMD was selected from one centre	No	Yes	Diagnosis only	Yes
Cystoid macular oedema	McBain *et al.*[100]	A series of patients was selected from an autofluorescence imaging database of one ophthalmology centre	No	Yes	Diagnosis only	No
	Dinc *et al.*[83]	Patients were selected from the 'FAF database' (no details provided) during a specified time period	Yes[a]	Not reported	Diagnosis only	No
Diabetic macular oedema	Vujosevic *et al.*[98]	Patients were recruited from diabetic retinopathy clinics (number not reported) during a specified time period	Unclear	Yes	Diagnosis only	No
	Waldstein *et al.*[84]	Chart review of all patients with diabetic retinopathy who underwent relevant imaging in one clinic during a specified time period	No	Yes	Diagnosis only	Yes

CFP, colour fundus photography.
a Informed consent was sought from all participants, suggesting the study was probably prospective.

Although FAF imaging may be used to detect autofluorescence in a wide range of retinal diseases (see *Chapter 1*), the eight included studies[83,84,96–101] cover only three broad retinal conditions. These are AMD (four studies[96,97,99,101]) cystoid macular oedema (two studies[83,100]) and diabetic macular oedema (two studies[84,98]). Of four studies on AMD, three studies specifically investigated FAF imaging in the detection of reticular pseudodrusen[96,97,101] and one study investigated FAF imaging in the diagnosis of choroidal neovascularisation.[99]

The eight studies included in the systematic review provide information on diagnostic accuracy only (see *Table 2*). No studies of the use of FAF imaging for monitoring the natural progression of retinal conditions, or for monitoring the response of retinal conditions to therapy, met the inclusion criteria.

Four of the studies involved the analysis of retrospective data,[84,97,100,101] two involved prospective recruitment of patients,[96,99] one appeared to prospectively recruit patients, although this was not explicitly reported,[83] and it is unclear whether the remaining study was retrospective or prospective[98] (see *Table 2*). Four of the studies selected patients consecutively (i.e. in the chronological order in which they first presented to clinicians)[84,97,98,100] and the remaining studies did not report whether selection was consecutive.[83,96,99,101] Retrospective studies, and those lacking consecutive patient recruitment, may be at greater risk of selection bias, and this is considered further below in relation to the overall assessment of the quality of evidence (see *Quality of research available*).

Most of the studies were conducted in Europe. The study by McBain and colleagues[100] was exclusively in the UK and the study by Waldstein and colleagues[84] also appears to have been conducted in the UK, although this was not explicitly reported. Two studies were conducted in multiple countries, which included the UK. Hogg and colleagues[96] conducted studies in Italy, Portugal and the UK, and Smith and colleagues[101] in the USA and UK. The remaining studies were conducted in Italy,[98] Portugal,[99] Turkey[83] and Japan.[97] The studies were published between 2006 and 2014. The earliest reported participant recruitment was in 2004[100] and the latest in November 2010[84,97] but dates of recruitment were not reported in three studies.[96,99,101]

Both men and women were included in the studies. Where reported (in seven studies[83,84,96–100]), the proportion of men ranged from 50% to 69%. Age was not reported in one study on AMD,[101] but in the remaining studies participants were older in the AMD studies (mean age 74–76 years)[96,97,99] than in the studies on cystoid macular oedema (mean age 59–62 years)[83,100] and diabetic macular oedema (mean age 49–67 years)[84,98] (see *Table 3*).

As indicated in *Table 3*, three of the four studies on AMD excluded participants with ocular comorbidities,[96,97,99] but this was not reported in the fourth study.[101] One study on cystoid macular oedema excluded participants with ocular comorbidities,[83] while the other did not report exclusion criteria.[100] For diabetic macular oedema, one study excluded patients with significant media opacities[98] while the other excluded patients with any macular comorbidity.[84]

In all studies the unit of analysis was the eye, and the number of eyes included ranged from 34 to 263 (see *Table 3*). As shown in *Table 4*, the studies differed according to whether they included one eye per patient,[96,99,100] both eyes,[97] or a mixture of single eyes and both eyes.[83,84,98,101] In the study on AMD by Smith and colleagues,[101] the results of two case series are reported (obtained from two databases – one in the USA and one in the UK) each with different inclusion criteria, one of which included both eyes, while the other included single eyes. However, the data on test accuracy from the study by Smith and colleagues[101] relevant to the current review are only available for the pooled population.

Descriptions of the retinal conditions varied considerably in detail and the studies were generally heterogeneous in terms of the conditions they included (see *Table 4*). The three studies on reticular pseudodrusen in AMD[96,97,101] differed according to the stage of AMD and whether it affected one or both eyes. The two studies on cystoid macular oedema[83,100] differed in the primary conditions from which the cystoid macular oedema developed. The studies on diabetic macular oedema[84,98] differed on whether they included ocular comorbidities.

TABLE 3 Patient characteristics

Retinal condition	Study	Country	Number of patients	Number of eyes	Mean age (range), years	% male	Ocular comorbidities permitted
AMD: choroidal neovascularisation	Cachulo *et al.*[99]	Portugal	52[a]	52	76 (56–92)	50	No
AMD: reticular pseudodrusen	Hogg *et al.*[96]	Italy, Portugal, UK	105	105	76 (52–93)	50	No
	Smith *et al.*[101]	UK, USA	138	221	Not reported	Not reported	Unclear
	Ueda-Arakawa *et al.*[97]	Japan	114	220	74 (52–92)	69	No
Cystoid macular oedema	McBain *et al.*[100]	UK	34	34	59 (17–89)	59	Yes
	Dinc *et al.*[83]	Turkey	55	67	62 (not reported)	51	Yes – apart from specific exclusions
Diabetic macular oedema	Vujosevic *et al.*[98]	Italy	137	263	T1D: 49 (28–64);[b] T2D: 67 (41–85)[b]	64	Yes – except significant media opacities
	Waldstein *et al.*[84]	UK? (not reported)	71	125	63 (not reported)	65	No

T1D, type 1 diabetes mellitus; T2D, type 2 diabetes mellitus.
a There were 62 eyes in total but only 52 were included in analyses.
b Not reported for all participants.

TABLE 4 Distribution and definition of the retinal conditions

Retinal condition	Study	Eye(s) studied (one or both) and definition of the condition
AMD: choroidal neovascularisation	Cachulo *et al.*[99]	Fellow eyes (defined as having early age-related maculopathy) of patients who had neovascular AMD in the non-study eye. Study eyes had: ≥ 5 intermediate (> 63 µm) or one large soft druse (> 125 µm), and/or confluent drusen within 3.0 µm of the foveal centre, with or without pigmentary changes
AMD: reticular pseudodrusen	Hogg *et al.*[96]	Fellow eyes of patients who had advanced unilateral neovascular AMD in the non-study eye. Study eyes specifically had early AMD with no features of GA or neovascular AMD
	Smith *et al.*[101]	Mixture of 83 pairs of eyes which had bilateral large, soft drusen (with or without GA) but had no evidence of choroidal neovascularisation, and 55 single eyes which were the fellow eyes of patients who had unilateral choroidal neovascularisation
	Ueda-Arakawa *et al.*[97]	Both eyes of patients newly diagnosed with early AMD, neovascular AMD or GA in at least one eye. Early AMD was defined as the presence of soft drusen (≥ 63 µm) or areas of hyper- or hypopigmentation in the retinal pigment epithelium, and GA was defined using colour fundus photography as a sharply delineated area (≥ 175 µm) of hypopigmentation, depigmentation or apparent absence of the retinal pigment epithelium in which choroidal vessels were clearly visible. Neovascular AMD was defined as neovascularisation detected using fluorescein or indocyanine green angiography
Cystoid macular oedema	McBain *et al.*[100]	Single eyes of patients with clinically suspected cystoid macular oedema secondary to cataract extraction, inherited retinopathies, inflammatory eye disease or idiopathic cases. In bilateral cases the left eye was arbitrarily chosen
	Dinc *et al.*[83]	Single eyes of 43 patients and both eyes of 12 patients whose cystoid macular oedema was secondary to diabetic retinopathy, retinal vein occlusions, uveitis, cataract surgery, epiretinal membrane formation or AMD
Diabetic macular oedema	Vujosevic *et al.*[98]	Both eyes of 126 patients and single eyes of 11 patients with diabetes mellitus (type 1 or 2) who had any stage of treated or untreated diabetic retinopathy
	Waldstein *et al.*[84]	Both eyes of 54 patients and single eyes of 17 patients with diabetic retinopathy with or without diabetic macular oedema

In keeping with the inclusion criteria for the current review, all FAF imaging tests were conducted using confocal scanning ophthalmoscopy (*Table 5*). The studies (except one that did not specify the image acquisition equipment[101]) used variants of the Heidelberg Retina Angiograph (Heidelberg Engineering, Heidelberg, Germany). Where reported (six studies[83,84,97–100]), the excitation wavelength was 488 nm (blue) and, where reported (five studies[83,97–100]), the detection wavelength was > 500 nm. Two of the studies included an additional FAF imaging test with a higher excitation wavelength; these were at 514 nm (green) in a study of diabetic macular oedema[84] and at 790 nm (near infrared) in a study of reticular pseudodrusen.[97] The field of view, which was specified in five studies,[83,84,96,99,100] was 30 degrees and the number of frames from which the final (mean) FAF image was calculated (specified in all studies except one[101]) ranged from 9 to 20.

TABLE 5 Test characteristics and diagnostic criteria

Retinal condition	Study	Index test	Index test diagnostic criteria (full details in *Appendix 5*)	Reference standard
AMD: choroidal neovascularisation	Cachulo *et al.*[99]	FAF: excitation 488 nm; detection > 500 nm (used HRA 2 cSLO)	Five types of FAF patterns. Images classified according to the International Fundus Autofluorescence Classification Group.[45,46] No other details provided	FA – stated as being the gold standard for assuming the presence of conversion of early age-related maculopathy to AMD (diagnostic criteria not reported)
AMD: reticular pseudodrusen	Hogg *et al.*[96]	FAF: excitation and detection wavelengths not stated (used Spectralis HRA + OCT cSLO)	Reticular hypoFAF pattern (identified using automated background levelling and segmentation): clusters of ill-defined hypoautofluorescent lesions interspersed against a background of mildly increased autofluorescence occurring in a regular and well-defined array	Positive diagnosis on one or more of five imaging modalities (CFP, RFP, IRP, FAF, OCT). Diagnostic criteria reported for each modality (see *Appendix 5*)
	Smith *et al.*[101]	FAF: excitation and detection wavelengths not stated (equipment not stated but cSLO implied)	Reticular hypoFAF pattern: stated only that definition excluded atrophic drusen patterns that were more central, darker and more scattered than the lower-contrast reticular autofluorescence that filled a whole region homogeneously	CFP (diagnostic criteria not explicitly reported – implied only that identification of drusen was based on areas of hypo- or hyperpigmentation)
	Ueda-Arakawa *et al.*[97]	(1) FAF: excitation 488 nm; detection > 500 nm (2) NIR-FAF: excitation 790 nm; detection not stated (used Spectralis HRA + OCT cSLO)	Reticular hypoFAF pattern: a group of ill-defined, hypofluorescent lesions against a background of mildly elevated autofluorescence	Positive diagnosis on two or more of seven imaging modalities (blue-channel CFP, IRR, FAF, NIR-FAF, CBR, ICGA, SD-OCT). Diagnostic criteria reported for each modality (see *Appendix 5*)

continued

TABLE 5 Test characteristics and diagnostic criteria (*continued*)

Retinal condition	Study	Index test	Index test diagnostic criteria (full details in *Appendix 5*)	Reference standard
Cystoid macular oedema	McBain *et al.*[100]	FAF: excitation 488 nm; detection > 500 nm (used HRA cSLO)	Round or oval areas of FAF at the fovea, with an autofluorescence signal similar to background levels (normal eyes would have hypoautofluorescence at this location due to luteal pigment blocking)	FA – stated used as the reference test as it has been used routinely for diagnosis of CMO; diagnosed if late-phase FA gave a petaloid dye leakage pattern around the fovea
	Dinc *et al.*[83]	FAF: excitation 488 nm; detection > 500 nm (used HRA cSLO)	Not reported explicitly but mentioned post hoc (in results section) as being round and oval hyperFAF at the fovea	FA – diagnosis of CMO made if late-phase FA showed pathognomonic leakage of fluorescein at the fovea in a petaloid configuration with feathery margins
Diabetic macular oedema	Vujosevic *et al.*[98]	FAF: excitation 488 nm; detection > 500 nm (used HRA 2 cSLO)	Single or multiple spot hyper FAF, no other details provided	RM-SLO (diagnostic criteria not reported)
	Waldstein *et al.*[84]	1. FAF: excitation 488 nm 2. FAF: excitation 514 nm; detection wavelengths not reported (used modified HRA classic cSLO) 3. MPOD mapping (not considered in the current report – see *Appendix 5*)	Not reported explicitly but mentioned post hoc (in results section) that DMO typically stands out as relatively bright, single or multiple, round or oval areas, mostly bordered by darker rims	SD-OCT – stated that this was considered the clinical standard for non-invasive diagnosis of DMO; only mentioned that signs of DMO on SD-OCT were intraretinal cysts, subfoveal neurosensory detachment and retinal thickening

CBR, confocal blue reflectance; CFP, colour fundus photography; CMO, cystoid macular oedema; DMO, diabetic macular oedema; FA, fluorescein angiography; HRA, Heidelberg Retina Angiograph; ICGA, indocyanine green angiography; IRP, infrared photography; IRR, infrared reflectance; MPOD, macular pigment optical density; OCT, optical coherence tomography; RFP, red fundus photography; RM-SLO, retromode scanning laser ophthalmoscopy; SD-OCT, spectral-domain optical coherence tomography.

The reference standards reported in the primary studies were: fluorescein angiography for diagnosing choroidal neovascularistion[99] and cystoid macular oedema;[83,100] colour fundus photography[101] or multiple imaging modalities[96,97] for diagnosing reticular pseudodrusen; and either retromode scanning laser ophthalmology[98] or spectral-domain optical coherence tomography[84] for diagnosing diabetic macular oedema (see *Table 5*). In the two studies that employed multiple imaging modalities,[96,97] the reference standard is somewhat unusual since diagnosis required a positive result on a specified minimum number of modalities. The clinical relevance of the reference standards is discussed further in our assessment of study quality (see *Quality of research available*).

Six of the studies reported that patients underwent an ophthalmic examination, which included assessments of visual acuity, intraocular pressure and/or slit lamp biomicroscopy[83,84,96–99] but they did not specify whether or not information obtained from these examinations was necessary to assist any of the diagnostic decisions made using the index test or reference standard. Three studies employed further retinal imaging tests in addition to their index test and reference standard[83,98,99] (these are listed as 'comparators' in *Appendix 5*). Two of these, by Cachulo and colleagues[99] on choroidal neovascularisation (where the additional tests were colour fundus photography, indocyanine green angiography, optical coherence tomography and a retinal leakage analysis) and by Dinc and colleagues[83] on cystoid macular oedema (where the additional test was

optical coherence tomography), did not explain whether or not any information from the additional tests was taken into account when making diagnostic decisions using FAF imaging or the reference standard (fluorescein angiography). The third study, by Vujosevic and colleagues[98] on diabetic macular oedema (where the additional tests were time-domain optical coherence tomography and fluorescein angiography), mentioned that images were graded in a masked fashion, suggesting that the additional tests would not have influenced any diagnostic decisions made using FAF imaging or the reference standard (retromode scanning laser ophthalmoscopy). One of the studies on diabetic macular oedema which employed both 488 nm and 514 nm wavelengths of FAF imaging used the results of both tests to calculate macular pigment optical density maps.[84] This approach was reported to have lower sensitivity and specificity for detecting diabetic macular oedema than either of the individual FAF imaging tests on which it was based (see *Appendix 5*) and as such is not considered as a separate index test in the current report.

The diagnostic criteria on FAF imaging for each of the retinal conditions were qualitative (i.e. descriptive) in all studies (see *Table 5*), and only one of the studies reported an objective (quantitative) approach for determining how abnormal (hypo or hyper) autofluorescence was defined.[101] The three studies on reticular pseudodrusen all specified that the diagnostic criterion was a reticular pattern of hypoautofluorescence, although there were slight differences in how this was described in each study.[96,97,101] The two studies on cystoid macular oedema[83,100] both appeared to diagnose the condition as round or oval patterns of autofluorescence at the fovea, although this was not stated explicitly in one study.[83] The two studies on diabetic macular oedema[84,98] only mentioned the diagnostic criteria briefly and differed in how these were described. Despite the subjective nature of the diagnostic criteria, only three of the eight studies investigated intergrader agreement in the classification of retinal images (see *Table 2*).[84,97,98] Limited information was provided about the expertise of the image graders, who were described as two independent ophthalmologists,[97] two retinal specialists trained in image grading[98] and two independent graders.[84]

Quality of research available

This section presents an overview of study rigour as assessed using the Cochrane adaptation[81] of the QUADAS tool.[93] *Table 6* summarises the critical appraisal judgements for each of the eight included studies.[83,84,96–101] Supporting explanations for the judgements are given in the full versions of the data extraction forms (see *Appendix 5*). As explained above (see *Table 1*) each of the QUADAS questions relates to a different aspect of bias or applicability that could limit the validity of the study results. An overview of these risks of bias is provided at the end of the section.

Quality assessment of diagnostic accuracy studies question 1: applicability of the patient spectrum

The populations included in the primary studies were considered unlikely to be representative of those who would present in NHS clinical practice (the patient spectrum was considered 'probably not representative' in seven studies[83,84,96,97,99,100,101] and unclear in one study[98]) (see *Table 6*). In the studies by Cachulo and colleagues,[99] Hogg and colleagues[96] and Smith and colleagues[101] the case mix was considered atypical and unlikely to represent patients in the NHS as the retinal condition was required to differ between the two eyes. In the studies by McBain and colleagues,[100] Dinc and colleagues,[83] Ueda-Arakawa and colleagues[97] and Waldstein and colleagues[84] the case mix also appears to have limited relevance to current NHS practice, as McBain and colleagues[100] and Dinc and colleagues[83] required cystoid macular oedema to be specific to certain causal conditions, while Ueda-Arakawa and colleagues[97] and Waldstein and colleagues[84] excluded patients with ocular comorbidities. In addition, the studies by McBain and colleagues,[100] Ueda-Arakawa and colleagues[97] and Waldstein and colleagues[84] selected patients retrospectively, which may further limit their applicability to current practice (as the case mix is potentially selective).[102] The study by Vusojevic and colleagues[98] included patients with any stage of treated or untreated diabetic retinopathy and permitted ocular comorbidities and, as such, appears potentially relevant to the NHS; however, the study was conducted in Italy and it is unclear whether patients were selected prospectively or retrospectively.

TABLE 6 Overview of QUADAS assessments

QUADAS question		CNV in AMD	Reticular pseudodrusen in AMD			Cystoid macular oedema		Diabetic macular oedema	
		Cachulo *et al.*[99]	Smith *et al.*[101]	Hogg *et al.*[96]	Ueda-Arakawa *et al.*[97]	Dinc *et al.*[83]	McBain *et al.*[100]	Vujosevic *et al.*[98]	Waldstein *et al.*[84]
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	No	No	No	No	No	No	Unclear	No
2	Is the reference standard likely to classify the target condition correctly?	Yes	Yes	Unclear	Unclear	Yes	Yes	Unclear	Yes
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes
5	Did patients receive the same reference standard irrespective of the index test result?	Yes	Yes	No	No	Yes	Yes	Yes	Yes
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	Yes	Yes	No	No	Yes	Yes	Yes	Yes
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Unclear
8	Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Unclear
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
10	Were uninterpretable/intermediate test results reported?	Yes[a]	No	No	Yes[b]	No	Yes[c]	No	No
11	Were withdrawals from the study explained?	Yes	Not applicable	No	Yes	Not applicable	Yes	Not applicable	Not applicable

CNV, choroidal neovascularisation.
a Poor image quality in two eyes (4%), but unclear whether or not these were included in analyses.
b Eighty-four eyes (38%) were excluded owing to poor image quality.
c Nine eyes (9%) were excluded owing to media opacities (cataracts).

Quality assessment of diagnostic accuracy studies question 2: applicability of reference standards

In five of the studies[83,84,99–101] the reference standards employed for diagnosing the target retinal condition were judged to be appropriate in principle as they are accepted standard methods: fluorescein angiography was used for diagnosing choroidal neovascularisation[99] and cystoid macular oedema;[83,100] colour fundus photography was used for diagnosing reticular pseudodrusen;[101] and spectral-domain optical coherence tomography was used for diagnosing diabetic macular oedema.[84] However, there are caveats around this interpretation because clinical experts advised that it is unlikely that individual imaging tests would be used in isolation, as appeared to be the case in these primary studies. For instance, fluorescein angiography would typically be employed in conjunction with optical coherence tomography when diagnosing cystoid macular oedema and diabetic macular oedema.[34,38] For the detection of reticular pseudodrusen, although colour fundus photography has been commonly used and is often considered a standard approach, this may not capture all the reticular pseudodrusen that could be identifiable using other imaging methods.[11,103] As noted in *Table 6*, for three studies we judged the ability of the reference standard to diagnose the target retinal condition as unclear.[96–98] This was either because the studies employed a suite of imaging modalities and required a positive result on a specified minimum number of these[96,97] (i.e. a non-standard diagnostic approach) or because the reference standard employed in the primary study (retromode scanning laser ophthalmology) has not been previously explored as a diagnostic tool for the specified condition (diabetic macular oedema).[98] As such, these three studies would not be reflective of standard imaging approaches employed in the NHS.

Quality assessment of diagnostic accuracy studies question 3: test interval

The time period between the index and reference tests (QUADAS question 3) was unclear (not reported) in five of the studies[83,96,97,99,101] and hence the risk of disease progression bias in these studies is unclear. In one study on cystoid macular oedema[100] and both studies on diabetic macular oedema[84,98] the time interval was considered short enough that both tests would have been assessing the same disease state and so these studies would be considered at low risk of disease progression bias.

Quality assessment of diagnostic accuracy studies question 4: population receiving the reference standard

In all studies except one[96] all the patients included in each study received the reference standard. In the study by Hogg and colleagues[96] on reticular pseudodrusen, multiple imaging methods were used, but it is unclear, according to the reported sample sizes, whether all participants received all tests and some data were then excluded, or whether some participants did not receive all tests.

Quality assessment of diagnostic accuracy studies question 5: same reference standard for all patients

In six studies all the patients included in each study received the same reference standard.[83,84,98–101] In the two studies where this was not the case, conducted by Hogg and colleagues[96] and Ueda-Arakawa and colleagues,[97] the combination of imaging modalities that contributed to a diagnosis (at least one of five tests[96] or at least two of seven tests[97]) could have differed between the patients.

Quality assessment of diagnostic accuracy studies question 6: independence of index test and reference standard

In six studies the index text was independent of the reference standard.[83,84,98–101] In the two studies where this was not the case, conducted by Hogg and colleagues[96] and Ueda-Arakawa and colleagues,[97] the index tests were among five tests[96] or seven tests[97] that made up the reference standard.

False positives are not possible where FAF imaging is also part of the reference standard and, as would be expected, no false positives were reported for FAF imaging by Hogg and colleagues.[96] However, Ueda-Arakawa and colleagues did report false positives (9/217 for FAF imaging and 5/136 for near-infrared FAF imaging). These false positives appear to be an artefact of the diagnostic criterion for reticular pseudodrusen, which required a positive result on two or more of seven imaging modalities. The ‘false

positives' in this case indicate that, in nine and five eyes, FAF imaging and near-infrared FAF imaging, respectively, were the only tests out of seven in the reference standard that detected reticular pseudodrusen.

Quality assessment of diagnostic accuracy studies question 7: interpretation of reference standard independent of index test results

In all studies except one[98] it was unclear (not reported) whether or not investigators interpreting the results of the reference standard test might have been aware of the results of the index test. In the study by Vusojevic and colleagues[98] the order of tests was not reported but it was stated that images were independently graded in a masked fashion.

Quality assessment of diagnostic accuracy studies question 8: interpretation of index test independent of reference standard results

In six studies it was unclear (not reported) whether or not investigators interpreting the results of the index test might have been aware of the results of the reference standard.[84,96,97,99–101] In the study by Dinc and colleagues[83] interpretation of the index test was independent of the reference standard as the index test was conducted first. In the study by Vusojevic and colleagues,[98] the order of tests was not reported but it was stated that images were independently graded in a masked fashion.

Quality assessment of diagnostic accuracy studies question 9: clinical information available for test interpretation

None of the studies explicitly stated whether or not their index tests or reference standards were interpreted in conjunction with other clinical information (i.e. the results from standard ophthalmic examinations or other imaging tests) and this casts considerable doubt over whether or not any of the reference standard tests as employed in the primary studies would properly represent how the tests would be employed in clinical practice.

Quality assessment of diagnostic accuracy studies question 10: reporting of uninterpretable or intermediate test results

Five of the studies (two on reticular pseudodrusen,[96,101] two on diabetic macular oedema[84,98] and one on cystoid macular oedema[83]) did not report whether uninterpretable or intermediate test results were considered either in the calculation of diagnostic accuracy or as exclusion criteria, so it is unclear whether or not image quality might have limited the utility of any of the imaging tests in these studies. Three studies reported poor quality or uninterpretable images.[97,99,100] Cachulo and colleagues[99] mentioned that the pattern of autofluorescence could not be determined for two eyes (4%) because of poor-quality images, but it is unclear whether or not these were included in the diagnostic accuracy calculation. Ueda-Arakawa and colleagues[97] reported that 84 eyes (38%) were excluded from analysis because of poor image quality. McBain and colleagues[100] stated that nine eyes (9%) were excluded because of poor FAF images.

Quality assessment of diagnostic accuracy studies question 11: study withdrawals explained

In four studies there were no withdrawals and so this question was answered as 'not applicable'.[83,84,98,101] In three studies the authors provided explanations for the withdrawals.[97,99,100] Cachulo and colleagues[99] reported these as death, withdrawal of informed consent, hospitalisation and loss to follow-up. McBain and colleagues[100] reported these as no FAF, more than 2 weeks between tests, FAF imaging < 4 days after the reference standard (no explanation provided), poor FAF images related to media opacities and cystoid macular oedema related to other diseases (e.g. previous branch vein occlusion, diabetic retinopathy or AMD). Ueda-Arakawa and colleagues[97] reported these as due to phthisis bulbi or poor image quality in three or more imaging modalities. One study, by Hogg and colleagues,[96] explained that images were excluded from analysis because of poor image quality but did not provide this information specifically for the index test.

Summary of quality assessment in relation to risks of bias

Seven of the eight included studies appear to be at high risk of spectrum bias (QUADAS question 1)[83,84,96,97,99–101] since their patient populations appeared not to be representative of those likely to be encountered in clinical practice, and in the remaining study[98] the risk of spectrum bias is unclear. Although most (five) of the studies appear to have employed reference standards that are commonly used and therefore might be considered acceptable (QUADAS question 2),[83,84,99–101] these studies appeared to employ single imaging tests as the reference standard whereas diagnostic decisions in clinical practice would more likely employ a combination of imaging tests. For this reason the risk of verification bias was considered as being unclear for these five studies and high for the remaining three studies[96–98] where the reference standard was unlikely to have accurately diagnosed the target retinal condition. The risk of disease progression bias (assessed in QUADAS question 3) is largely unclear, except for one study on cystoid macular oedema[100] and both studies on diabetic macular oedema,[84,98] which were judged to be at low risk. The risk of differential verification bias, that is, bias arising as a result of participants not all receiving the same reference standard (assessed in QUADAS questions 4 and 5), was considered to be low for most (six)[83,84,98–101] of the studies but high for two studies[96,97] on reticular pseudodrusen owing an unorthodox design of reference standard (where diagnosis required a positive result on a specified minimum number of imaging modalities). These same two studies on reticular pseudodrusen were considered at high risk of incorporation bias (assessed in QUADAS question 6) since the index test was not independent of the reference standard; the remaining six studies were judged to have low risk of this bias.[83,84,98–101] The risk of diagnostic review bias, that is, the index test result influencing interpretation of the reference standard result (assessed in QUADAS question 7) was unclear for most (seven) studies but judged to be low for one study on diabetic macular oedema.[98] The risk of test review bias, that is, where results of the reference standard influence interpretation of the index test result (assessed in QUADAS question 8) was unclear in most (six) studies[84,96,97,99–101] but judged to be low for one study on cystoid macular oedema[83] and for one study on diabetic macular oedema.[98] The risk of clinical review bias (assessed in QUADAS question 9) is unclear since none of the included studies reported whether their index tests were interpreted together with other clinical information, such as the results of routine ophthalmic examinations or results of other imaging modalities they employed. The risk of bias from uninterpretable or intermediate test results (QUADAS question 10) is generally unclear. Risk of attrition bias (QUADAS question 11) was judged to be low in five studies since either there were no exclusions,[83,84,98,101] or the exclusions were clearly explained and appeared unlikely to influence the analysis.[97] Two studies explained their withdrawals but presented discrepancies in the numbers they analysed[99,100] (see *Appendix 5*), while one study did not explain withdrawals;[96] these three studies were judged to have an unclear risk of attrition bias. Overall, taking into consideration the different risks of bias mentioned above, it is not possible to identify individual studies, or specific retinal conditions, for which the quality of the clinical evidence would be regarded as being adequately robust to directly inform clinical practice.

An aspect of study quality not captured by the QUADAS criteria is that studies that included both eyes of a patient did not take into account adjustment for the correlation between the patient's eyes, and this might lead to underestimation of standard errors for sensitivity and specificity.[95] This applies to two studies of reticular pseudodrusen in AMD by Ueda-Arakawa and colleagues[97] and Smith and colleagues,[101] and to both studies of diabetic macular oedema, by Vusojevic and colleagues[98] and Waldstein and colleagues.[84]

Assessment of test accuracy

This section presents the best evidence available on the diagnostic accuracy of FAF imaging. Given the heterogeneity of study characteristics with respect to the retinal conditions included, whether they were assessed in one or both eyes and differences between studies in the reference standards employed (see *Quantity of research available*), it would be inappropriate to quantitatively pool diagnostic accuracy outcomes (i.e. sensitivity and specificity) across individual studies within each of the retinal conditions. Instead a narrative synthesis was undertaken, which provides sensitivity and specificity estimates for each retinal condition together with their confidence intervals, as well as likelihood ratios to illustrate the overall

diagnostic results from each study and their uncertainty. Paired sensitivity and specificity forest plots are presented for illustrative purposes, without pooling the sensitivity or specificity measures across the studies. For brevity, likelihood ratios are discussed below only in cases where they suggest that the results of a given study might not be diagnostically informative (based on the criteria given in *Chapter 2*). Diagnostic odds ratios and positive and negative predictive values are also available for all studies (see *Appendix 5*). However, these statistics have to be interpreted with caution.[89] Given that there are concerns about the quality of the evidence in all studies (see *Quality of research available*), and in the absence of a quantitative meta-analysis, the predictive values and diagnostic odds ratio would not provide additional interpretational value in the assessment of diagnostic accuracy and as such they are not referred to below.

Choroidal neovascularisation in neovascular age-related macular degeneration

One study, by Cachulo and colleagues,[99] assessed the diagnostic accuracy of FAF imaging (488 nm) against fluorescein angiography in detecting choroidal neovascularisation in AMD. The study authors reported sensitivity of 93% and specificity of 37%. However, using the data available from Cachulo and colleagues,[99] we calculated sensitivity of 88% and specificity of 34% (*Table 7*) (for calculations see *Appendix 5*). This discrepancy might result from differences in categorising two (out of 52) eyes for which the pattern of FAF could not be determined because of poor-quality images. Confidence intervals for the estimates of sensitivity and specificity suggest moderate uncertainty around these values (*Figure 3*), but were not reported for the estimates calculated by the study authors.

Cachulo and colleagues[99] did not report any information on interobserver or intraobserver agreement in image grading, test acceptability to patients or clinicians, or adverse events that might influence the interpretation of diagnostic outcomes.

As noted above (see *Quality of research available*), the single available study on diagnosis of choroidal neovascularisation in AMD[99] was judged to be at high risk of spectrum bias (owing to an unrepresentative case mix); in addition the risks of several types of bias that could arise in relation to the sequence and timing of the index and reference tests, as well as the risk of bias in the interpretation of these tests, were unclear. Taking these considerations into account, it is not possible to draw a rigorous clinically relevant conclusion about the diagnostic accuracy of FAF 488 nm imaging as compared against fluorescein angiography in this retinal condition.

TABLE 7 Diagnostic accuracy of FAF imaging for choroidal neovascularisation in neovascular AMD

Study	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive likelihood ratio, % (95% CI)	Negative likelihood ratio, % (95% CI)
Cachulo *et al.*, 2011[99]	93 (not reported)	37 (not reported)	Not reported	Not reported
Calculated by reviewer	88.24 (63.52 to 98.20)	34.29 (19.15 to 52.21)	1.34 (1.00 to 1.80)	0.34 (0.09 to 1.36)

CI, confidence interval.

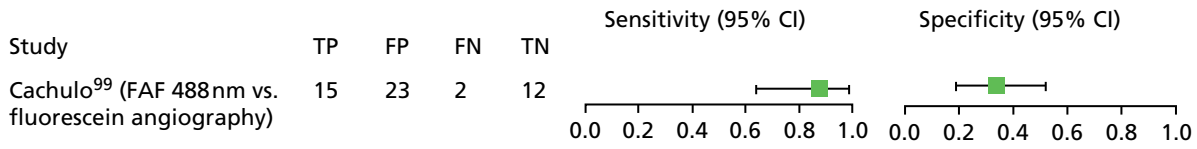

FIGURE 3 Diagnostic accuracy of FAF imaging for choroidal neovascularisation in neovascular AMD. CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Reticular pseudodrusen in age-related macular degeneration (different stages)

Three studies, by Hogg and colleagues,[96] Smith and colleagues[101] and Ueda-Arakawa and colleagues,[97] assessed the accuracy of FAF imaging for detecting reticular pseudodrusen in AMD.

Two of the studies did not report the FAF imaging excitation wavelength,[96,101] while Ueda Arakawa and colleagues[97] used FAF 488 nm and infrared FAF 790 nm (NIR-FAF) as index tests. The studies used various reference standards. Smith and colleagues[101] used colour fundus photography, Hogg and colleagues[96] required a positive result on one or more of five imaging modalities, and Ueda-Arakawa and colleagues[97] required a positive result on two or more of seven imaging modalities. Each study aimed to detect reticular pseudodrusen in a different stage of AMD (see *Table 4*). Hogg and colleagues[96] studied fellow eyes of patients with neovascular AMD in the non-study eye, Ueda-Arakawa and colleagues[97] studied both eyes of patients newly diagnosed with early AMD, neovascular AMD or GA, and Smith and colleagues[101] included a mixture of both eyes of patients without any evidence of choroidal neovascularisation and fellow eyes of patients with unilateral choroidal neovascularisation in the non-study eye.

By taking together the appropriate reference standards for diagnosing reticular pseudodrusen (i.e. colour fundus photography and optical coherence tomography) and the other reference standards nominated in the primary studies, data are available for six diagnostic accuracy comparisons for FAF imaging (*Table 8*). Although the reference standard reported by Hogg and colleagues[96] involves a positive result on one or more tests, individual comparisons of FAF imaging against colour fundus photography and FAF imaging against optical coherence tomography were also reported.

TABLE 8 Diagnostic accuracy of FAF imaging for reticular pseudodrusen in AMD

Study	Index text	Reference standard	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive likelihood ratio, % (95% CI)	Negative likelihood ratio, % (95% CI)
Smith *et al.*[101]	FAF[a]	CFP	93.33 (77.89 to 98.99)	97.91 (94.72 to 99.41)	44.57 (16.82 to 118.08)	0.07 (0.02 to 0.26)
Hogg *et al.*[96]	FAF[a]	Spectralis OCT	87.88 (71.78 to 96.52)	84.21 (72.13 to 92.30)	5.57 (3.02 to 10.27)	0.14 (0.06 to 0.36)
		CFP	100 (78.03 to 100.00)	66.67 (55.08 to 76.94)	3.00 (2.19 to 4.11)	0.00 (not calculable)
		Positive on one of more of five imaging modalities (CFP, RFP, IRP, FAF, OCT)	95.35 (84.16 to 99.30)	100 (92.82 to 100.00)	Not calculable	0.05 (0.01 to 0.18)
Ueda-Arakawa *et al.*[97]	FAF 488 nm	Positive on two or more of seven imaging modalities (CFP, IRR, FAF, NIR-FAF, CBR, ICGA, SD-OCT)	86.49 (71.21 to 95.41)	95.00 (90.72 to 97.68)	17.30 (9.04 to 33.11)	0.14 (0.06 to 0.32)
	FAF 790 nm (NIR-FAF)		32.14 (15.91 to 52.35)	95.37 (89.52 to 98.46)	6.94 (2.53 to 19.08)	0.71 (0.55 to 0.92)

CBR, confocal blue reflectance; CFP, colour fundus photography; CI, confidence interval; ICGA, indocyanine green angiography; IRP, infrared photography; IRR, infrared reflectance; OCT, optical coherence tomography; RFP, red fundus photography; SD-OCT, spectral domain optical coherence tomography.
a Excitation wavelength not stated.

FAF imaging based on a 488 nm excitation wavelength had relatively high (86–100%) sensitivity for detecting reticular pseudodrusen when compared against the various reference standards (*Figure 4*). However, near-infrared FAF imaging had low (32%) sensitivity (see *Figure 4*). In contrast, both FAF and NIR-FAF imaging had high (84–100%) specificity compared against the various reference standards, except for a lower specificity (67%) when FAF imaging was compared against colour fundus photography in the study by Hogg and colleagues.[96] A major limitation of the results for near-infrared FAF imaging reported by Ueda-Arakawa and colleagues[97] is that 38% of the eyes were excluded owing to poor image quality.

For most of the studies the 95% confidence intervals around the specificity estimates are relatively narrow. However, confidence intervals for specificity reported by Hogg and colleagues,[96] and those reported for sensitivity by all studies are wider, meaning that these estimates may be less reliable (see *Figure 4*). However, it is possible that the width of the confidence intervals reported by Smith and colleagues[101] and Ueda-Arakawa and colleagues[97] might have been underestimated, as the analyses did not account for any intrapatient correlations in cases where both eyes of the same patient were analysed.

Two of the studies, by Hogg and colleagues[96] and Ueda-Arakawa and colleagues,[97] provided information on intergrader agreement in diagnosing reticular pseudodrusen using FAF imaging. Kappa values indicate actual agreement compared with that which would occur by chance. A value of 1 indicates perfect agreement and a value of 0 indicates agreement equivalent to chance. Kappa values were 0.563 ($n = 35$, $p < 0.001$) (84.2% agreement)[97] and 0.94 (sample size, p-value and percentage agreement not reported).[96] These kappa values could be interpreted as 'moderate' and 'almost perfect' agreement, respectively.[104] Although intergrader agreement was almost perfect in Hogg and colleagues' study,[96] it was reported for only one of their three study centres and so it is unclear whether or not the 'best' agreement was selectively presented or whether or not the reported agreement is reflective of that across all the study centres. None of the studies on reticular pseudodrusen provided any information on intragrader variation in diagnosis, the acceptability of the imaging modalities to patients or clinicians, or on whether any adverse events of any tests were observed.

In summary, the diagnostic outcomes suggest that FAF imaging with an excitation wavelength of 488 nm could potentially contribute to the diagnosis of reticular pseudodrusen but near-infrared FAF imaging (790 nm) does not appear adequately sensitive to be of diagnostic value. However, there is considerable uncertainty around these findings, owing to the limited number of studies and comparisons available and shortcomings in the study designs. As noted above (see *Quality of research available*), the three available studies on diagnosis of reticular pseudodrusen in AMD[96,97,101] were judged to be at high risk of spectrum

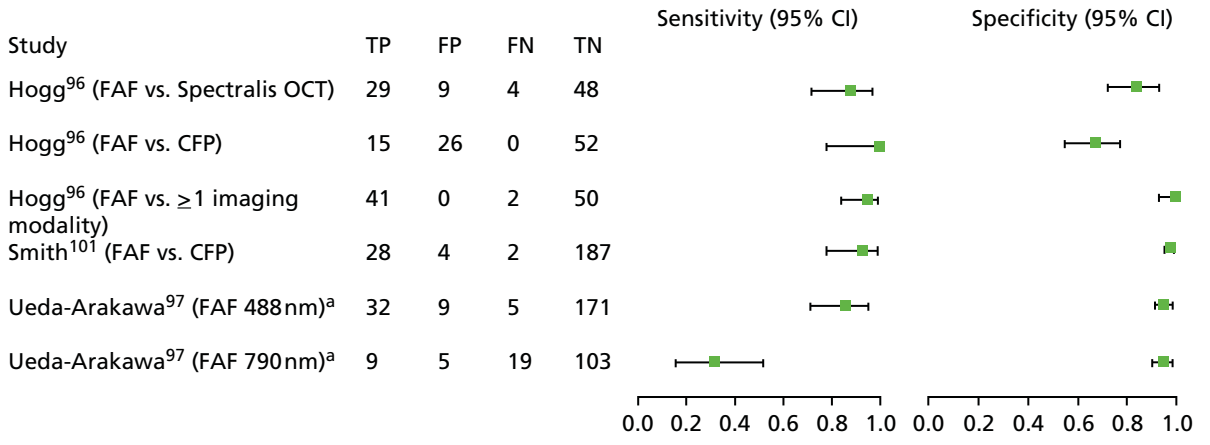

FIGURE 4 Diagnostic accuracy of FAF imaging for reticular pseudodrusen in AMD. CFP, colour fundus photography; CI, confidence interval; FN, false negative; FP, false positive; OCT, optical coherence tomography; TN, true negative; TP, true positive. a, Versus two or more imaging modalities.

bias (owing to an unrepresentative case mix), while the risks of several types of bias that could arise in relation to the sequence and timing of the FAF imaging tests in relation to those of the reference standards, and in the interpretation of these tests, were unclear. In addition, in the studies by Hogg and colleagues[96] and Ueda-Arakawa and colleagues,[97] the FAF imaging index tests also formed part of the reference standard, which complicates interpretation of any diagnostic outcomes. Furthermore, Ueda-Arakawa and colleagues[97] had to exclude over one-third of the eyes from their analysis of near-infrared FAF imaging because good image quality could not be obtained with this method. Taking these considerations into account, it is not possible to draw a rigorous clinically relevant conclusion about the diagnostic accuracy of FAF imaging for diagnosing reticular pseudodrusen in AMD.

Cystoid macular oedema secondary to various conditions

Two studies, by Dinc and colleagues[83] and McBain and colleagues,[100] reported the diagnostic accuracy of 488 nm FAF imaging for identifying cystoid macular oedema secondary to various conditions. Both studies employed fluorescein angiography as the reference standard, which would partly reflect current clinical practice (optical coherence tomography and possibly also colour fundus imaging would be used in addition to fluorescein angiography to reach a diagnosis in clinical practice). The study by Dinc and colleagues[83] indicated high (98%) sensitivity with a narrow confidence interval suggesting good precision of the estimate, while that of McBain and colleagues[100] indicated moderate sensitivity (81%) but with high uncertainty (*Table 9* and *Figure 5*). However, the former study had 0% specificity (reflecting a lack of true negatives in the data) and hence, as the likelihood ratios suggest, the study is not diagnostically informative. The latter study, by McBain and colleagues,[100] is limited by the uncertainty around its sensitivity and specificity estimates which, according to the lower confidence limits, could have been as low as 58% and 39%, respectively.

Neither of the two studies on cystoid macular oedema[83,100] reported any information on interobserver or intraobserver agreement in image grading, test acceptability to patients or clinicians, or adverse events of any tests that might influence the interpretation of diagnostic outcomes.

TABLE 9 Diagnostic accuracy of FAF imaging for cystoid macular oedema

Study	Index text	Reference standard	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive likelihood ratio, % (95% CI)	Negative likelihood ratio, % (95% CI)
Dinc *et al.*[83]	FAF 488 nm	FA	98.46 (91.69 to 99.74)	0.00 (0.00 to 80.71)	0.98 (0.96 to 1.01)	Not calculable
McBain *et al.*[100]	FAF 488 nm	FA	80.95 (58.08 to 94.44)	69.23 (38.61 to 90.72)	2.63 (1.13 to 6.10)	0.28 (0.11 to 0.71)

CI, confidence interval; CMO, cystoid macular oedema; FA, fluorescein angiography.

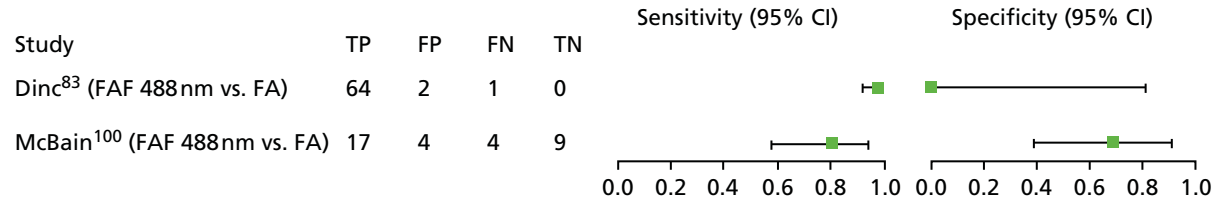

FIGURE 5 Diagnostic accuracy of FAF imaging for cystoid macular oedema. CI, confidence interval; FA, fluorescein angiography; FN, false negative; FP, false positive; TN true negative; TP, true positive.

As noted above (see *Quality of research available*), both studies on diagnosis of cystoid macular oedema[83,100] were judged to be at high risk of spectrum bias, owing to an unrepresentative case mix. Although Dinc and colleagues[83] interpreted results of the index test (FAF imaging) without knowledge of the results of the reference standard (fluorescein angiography), indicating a low risk of test review bias, the risks of diagnostic review bias and clinical review bias were unclear in both studies. Taking these considerations into account, noting that one study appears to be diagnostically uninformative[83] while the other has high uncertainty of its diagnostic outcomes,[100] it is not possible to draw a rigorous clinically relevant conclusion about the accuracy of FAF imaging for diagnosing cystoid macular oedema.

Diabetic macular oedema

Two studies, by Vujosevic and colleagues[98] and Waldstein and colleagues,[84] reported the diagnostic accuracy of FAF imaging for diabetic macular oedema. Vujosevic and colleagues[98] compared 488 nm FAF imaging against a reference standard of retromode scanning laser ophthalmoscopy. Waldstein and colleagues[84] compared 488 nm and 514 nm FAF imaging against a reference standard of spectral-domain optical coherence tomography. For diabetic macular oedema, optical coherence tomography would be a clinically relevant reference standard (as acknowledged by Vusojevic and colleagues[98]), although, according to clinical experts consulted during the present review, it would likely be combined in clinical practice with colour fundus imaging and, if macular ischaemia is suspected, also with fluorescein angiography.

Diagnostic outcomes are shown in *Table 10* and *Figure 6*. Sensitivity of FAF imaging (488 nm) for diagnosing diabetic macular oedema was highest (92%) when compared against retromode scanning laser ophthalmoscopy, although this is unlikely to be used routinely in clinical practice. Sensitivity of FAF imaging when compared against spectral-domain optical coherence tomography was moderate (81%) for the shorter-wavelength imaging (488 nm) but relatively low (55%) for the longer-wavelength method (514 nm).

TABLE 10 Diagnostic accuracy of FAF imaging for diabetic macular oedema

Study	Index text	Reference standard	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive likelihood ratio, % (95% CI)	Negative likelihood ratio, % (95% CI)
Vusojevic *et al.*[98]	FAF 488 nm	RM-SLO	92.42 (87.98 to 95.60)	84.62 (71.91 to 93.10)	6.01 (3.17 to 11.38)	0.09 (0.06 to 0.15)
Waldstein *et al.*[84]	FAF 488 nm	SD-OCT	80.60 (69.11 to 89.24)	89.66 (78.82 to 96.08)	7.79 (3.62 to 16.77)	0.22 (0.13 to 0.36)
	FAF 514 nm		55.22 (42.58 to 67.39)	94.83 (85.60 to 98.86)	10.68 (3.47 to 32.82)	0.47 (0.36 to 0.62)

CI, confidence interval; RM-SLO, retromode scanning laser ophthalmoscopy; SD-OCT, spectral domain optical coherence tomography.

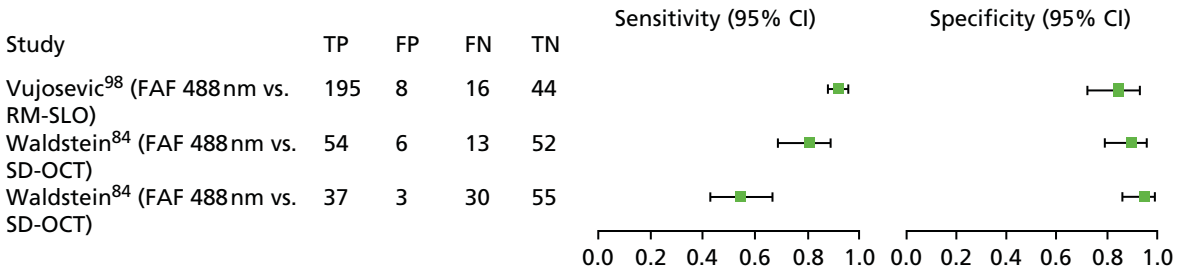

FIGURE 6 Diagnostic accuracy of FAF imaging for diabetic macular oedema. CI, confidence interval; FN, false negative; FP, false positive; RM-SLO, retromode scanning laser opthalmoscopy; SD-OCT, spectral domain optical coherence tomography; TN, true negative; TP, true positive.

In contrast, specificity was relatively high for both wavelengths of FAF imaging when compared against spectral-domain optical coherence tomography (90% to 95%) but lower when 488 nm FAF imaging was compared against retromode scanning laser ophthalmoscopy (85%). The estimates of sensitivity and specificity have relatively narrow confidence intervals, suggesting they are reasonably precise, although it is possible that the width of the confidence intervals for both studies might have been underestimated, since the analyses did not account for any intrapatient correlations where both eyes of the same patient were analysed.

Vusojevic and colleagues[98] did not report intergrader agreement on interpreting FAF images. Waldstein and colleagues[84] reported kappa values (but not sample size, *p*-value or percentage reviewer agreement) for intergrader agreement in interpretation of the FAF images, which were 0.84 for 488 nm FAF imaging and 0.63 for 514 nm FAF imaging. These could be interpreted as 'almost perfect agreement' and 'substantial agreement', respectively.[104] Neither study reported intraobserver agreement for image grading, test acceptability to patients or clinicians, or adverse events of any tests that might influence the interpretation of diagnostic outcomes.

As noted above (see *Quality of research available),* the study by Waldstein and colleagues[84] was judged to be at high risk of spectrum bias (owing to an unrepresentative and retrospectively selected case mix) while the risks of various types of bias associated with the timing of the index test and reference standard, and interpretation of these tests, were judged to be unclear. The study by Vujosevic and colleagues[98] was considered to be at unclear risk of spectrum bias because although the case mix appears potentially reflective of patients likely to receive retinal imaging in clinical practice (diabetic retinopathy patients with comorbidities permitted) it is unclear whether the patients were selected prospectively or retrospectively. This study[98] was the only one of the eight included in the systematic review that was deemed to be at low risk of both test review bias and diagnostic review bias. However, as noted above, the reference standard employed by Vusojevic and colleagues[98] is unlikely to reflect clinical practice. Taking these considerations into account, it is not possible to draw a rigorous clinically relevant conclusion about the accuracy of FAF imaging for diagnosing diabetic macular oedema, although results from the study by Waldstein and colleagues[84] suggest that the longer-wavelength FAF imaging method (514 nm) was less sensitive for diagnosing diabetic macular oedema than the standard wavelength approach (488 nm).

Summary of diagnostic accuracy assessment

The eight studies[83,84,96–101] included in the systematic review suggest qualitatively that FAF imaging may have a potential role in supporting the diagnosis of choroidal neovascularisation in AMD, reticular pseudodrusen in AMD, cystoid macular oedema or diabetic macular oedema. However, after taking into account a number of quality issues concerning the study designs, none of the studies were found to provide convincing quantitative information about the diagnostic accuracy of FAF imaging of relevance to current clinical practice. In two cases where long and short excitation wavelengths of FAF imaging could be compared, the longer wavelength appeared less diagnostically sensitive: 719 nm was less sensitive than 488 nm for detecting reticular pseudodrusen[97] while 514 nm was less sensitive than 488 nm for detecting diabetic macular oedema.[84] However, these differences are based on only two studies that had methodological limitations and, as such, are illustrative only.

Chapter 5 Discussion

Statement of principal findings

- There is considerable primary research interest in FAF imaging of retinal conditions (of 206 full-text papers retrieved for inspection, 199 papers reported on primary studies that had employed FAF imaging to investigate retinal conditions). However, most studies have been descriptive, providing reports of how FAF patterns reflect the pathology of retinal conditions (e.g.[105–107]), rather than quantitatively assessing diagnostic or monitoring accuracy.
- Eight primary research studies met the inclusion criteria for the current review.[83,84,96–101] These investigated the accuracy of FAF imaging in diagnosing choroidal neovascularisation (one study[99]), reticular pseudodrusen in various stages of AMD (three studies[96,97,101]), cystoid macular oedema (two studies[83,100]) and diabetic macular oedema (two studies[84,98]).
- No studies have assessed the accuracy of FAF imaging for monitoring the progression of retinal conditions or their response to therapy.
- Most of the studies included in the current review provided very limited information on the diagnostic criteria they used and these were qualitative (descriptive); most studies did not check for intergrader agreement in reading FAF images, despite the potential subjectivity of the descriptive diagnostic criteria; and none of the studies reported the clinical information used by researchers when interpreting FAF images.
- In most of the included studies that used an excitation wavelength of 488 nm, the sensitivity of FAF imaging was high (range 81–100%), although it was lower in two studies that used longer excitation wavelengths (32% in one study of reticular pseudodrusen in AMD which used an excitation wavelength of 790 nm,[97] and 55% in one study of diabetic macular oedema, which used an excitation wavelength of 514 nm[84]). The specificity of FAF imaging across all studies ranged from 34% to 100% and was not clearly related to the excitation wavelength. However, owing to the relative paucity of reliable data, and limitations in experimental rigour, it is unclear whether FAF imaging would be accurate in diagnosing retinal conditions or in monitoring their progression or response to therapy in clinical practice.

The current review has identified several evidence gaps, including:

- Robust data on the monitoring accuracy of FAF imaging for assessing the progression of retinal conditions or their response to therapy do not currently exist.
- Limited data are available on the diagnostic accuracy of FAF imaging but these are for four retinal conditions and suffer from methodological limitations. As such, they are effectively qualitative rather than reliably quantitative.
- No diagnostic or monitoring accuracy studies of FAF imaging were found for the inherited retinal dystrophies, early AMD, GA or CSC. According to the review advisory group, these are conditions where FAF imaging might be most useful and is already being used (to an unclear extent) in the NHS.

Methodological challenges

A challenge in the current review was to identify studies of the diagnostic accuracy of FAF imaging based on screening titles and abstracts. It was difficult to determine, based on titles and abstracts alone, whether or not studies reported sensitivity and specificity, or data from which these could be calculated. As a result it was necessary to retrieve and check 206 full-text records. Nevertheless, only eight studies finally met the inclusion criteria.[83,84,96–101] A difficulty is that most of these eight studies that provided relevant diagnostic outcomes were not primarily diagnostic studies and as such they were rated relatively poorly against the QUADAS criteria for risks of bias. Ideally, future studies that wish to explore the diagnostic accuracy of FAF imaging should be based on appropriate designs and methods compatible with the paradigm of diagnostic accuracy assessment,[108,109] and should include a clear statement in the abstract as to the availability of quantitative diagnostic outcomes.[110]

Patient and public involvement

This review did not formally involve patients or the general public. However, the draft protocol and report were provided to the advisory group, which included the RNIB, for comment. The RNIB did not identify any major issues of equitability in the use of FAF imaging from the perspective of patients and the general public. However, it should be noted that the technology is relatively new and the extent to which patients have access to the technology is currently unclear.

Strengths and limitations of the assessment

Strengths

- The review was based on a prespecified peer-reviewed protocol.
- A comprehensive literature search was conducted based on a wide range of prespecified evidence sources.
- All steps of the systematic review process were conducted by at least two reviewers, minimising the risks of errors and bias.
- The primary evidence was assessed critically using accepted criteria for the critical appraisal of test accuracy studies.
- All steps of the evidence synthesis are reported transparently; worksheets for study selection and data extraction were pilot tested and are presented with this report; all studies excluded at full-text screening are listed in *Appendix 4*, stating reasons for exclusion.
- An independent advisory group informed the review.

Limitations

- Interpretation of the primary research is hampered by clinical heterogeneity among the studies and limitations in methodological rigour, which in all studies led to judgements of high risk of bias. Owing to the small number of studies available it was not possible to assess the impact of methodological rigour on observed diagnostic outcomes.
- The primary research studies had an inappropriate (or in one study unclear[98]) patient spectrum (i.e. a case mix unlikely to be reflective of that presenting for retinal imaging in current NHS practice).
- Some studies included both eyes of patients in analyses, which (because of intrasubject correlations) may have led to underestimation of standard errors for sensitivity and specificity (and hence also underestimation of the width of the confidence intervals).[95] As there is no formally agreed approach for analysing such data in evidence syntheses,[95] the current review does not investigate their impact on the precision of diagnostic outcomes. However, such cases are clearly identified in the review and any inaccuracy of confidence intervals in these cases would not have markedly altered the review conclusions.
- The systematic review was limited to English-language studies.

Uncertainties

- The current use of FAF imaging for diagnosing and/or monitoring retinal conditions in clinical practice is unclear.
- The relevance of FAF imaging for monitoring retinal conditions is uncertain. Numerous studies suggest FAF imaging appears to have potential value as a monitoring tool (e.g. detecting the progression of the GA area in dry AMD, or the presence and distribution of autofluorescence and development of atrophy in different inherited retinal dystrophies), but so far all studies that have assessed FAF imaging as a monitoring tool have lacked a reference standard.
- The information required to adequately interpret FAF images in primary studies is unclear, as it was not reported in any study whether FAF images were interpreted in isolation or in conjunction with other clinical information. All studies were therefore judged to be at unclear risk of clinical review bias. This is important as diagnostic tests should ideally be interpreted using the same information that would be available in clinical practice.
- It is uncertain how variability between methods (different models of cSLO with different software and image acquisition protocols) may have affected diagnostic outcomes as this level of detail was not consistently reported in the included studies.
- It is uncertain whether or not diagnostic criteria used for FAF imaging are reliable (interobserver reliability of diagnostic criteria was assessed in only three out of eight studies and only in limited detail[84,96,97]). The recently updated classification of early AMD and GA using FAF, by the International Fundus Autofluorescence Classification group,[45,46] was used in one of the included studies.[99] However, the classification consists of descriptions of patterns seen on retinal imaging rather than quantifiable characteristics, and, as such, is open to subjective interpretation. There appears to be a lack of internationally identified and agreed classifications for other conditions, although these may be needed to allow meaningful interpretation in future diagnostic accuracy studies of FAF imaging.
- Although it was not an objective of the current review to assess costs or cost-effectiveness of FAF imaging, it was noted during the inspection of full-text articles that none of the studies that have assessed the diagnostic accuracy of FAF imaging provided any information on costs or resources, such as the time taken to acquire images or any resources used for image processing and quality control.

Chapter 6 Conclusions

It is not possible to give a clear indication of the diagnostic or monitoring accuracy of FAF imaging for retinal conditions based on existing research. Although some studies reported relatively high sensitivity, these had various methodological limitations, which hinder the interpretation of test accuracy. There is an indication that standard wavelength FAF imaging (488 nm) may be more sensitive than longer-wavelength approaches but this is based on only two studies, involving 790-nm imaging for detecting reticular pseudodrusen[97] and 514-nm imaging for detecting diabetic macular oedema.[84] Owing to the relative paucity of reliable data, further studies are required. In particular, prospective studies would be helpful in inherited retinal dystrophies, early AMD, GA and CSC, and the studies should be designed according to the paradigm for the quantitative assessment of test accuracy.

Implications for service provision

Owing to a lack of studies addressing the appropriate populations and employing appropriate imaging methods there remains uncertainty whether or not FAF imaging is accurate for the diagnosis and monitoring of retinal conditions in clinical practice. As noted in the specific recommendations below, research is needed to address these knowledge gaps.

The current extent of use of FAF imaging for different retinal conditions in the NHS is unclear. An audit or survey of current practice would be helpful to clarify how the method is used and whether or not its usage is appropriate, so as to inform future guidance for NHS practice. The review advisory group suggested that although the role of FAF imaging in clinical practice is still developing it may already be used by specialists in the NHS to assist with diagnosis and monitoring of inherited retinal dystrophies such as Stargardt disease, and may also have a role in the diagnosis and/or monitoring of early AMD and GA.

There seems to be lack of consensus on whether or not FAF imaging may have a role in diagnosing wet AMD or macular oedemas. Clinical experts advising the current review suggested that the oedema and fluid leakage in these conditions would obscure the FAF signal, meaning that both fluorescein angiography and optical coherence tomography would be more appropriate imaging modalities for making a diagnosis (since blood perfusion and leakage, and macular thickness are key diagnostic aspects of these conditions). However, some researchers have suggested that FAF imaging could have a role for diagnosing cystoid macular oedema[107] and retinal pigment epithelium loss in wet AMD,[67] while the studies on choroidal neovascularisation,[99] cystoid macular oedema[83,100] and diabetic macular oedema[84,98] that were included in the current review highlight that there is research interest in the use of FAF imaging in detecting and diagnosing, or monitoring these conditions.

Given that FAF imaging is non-invasive, there might be benefits to both patients and the NHS if FAF imaging could, in some cases, replace fluorescein angiography, which is the most frequently used invasive retinal imaging test. Fluorescein angiography carries a risk of complications (adverse reaction to dye), side effects (ranging from mild nausea or hives to anaphylaxis and death) and may impact on patients' quality of life (e.g. fear of needles), as well as requiring specialist clinical facilities (including a sterile clinical environment and staff qualified in venous access). Where complications occur or patients experience diminished quality of life this may require additional therapy, which would impact on health resources. None of the studies included in the current review assessed patients' perceptions of the test procedures or reported whether or not the angiography reference standard was associated with any adverse events. Further evidence would therefore be helpful to clarify the magnitude of benefits or disadvantages to patients and the NHS of any switch from fluorescein angiography to FAF imaging.

Any future research into the accuracy of FAF imaging (see *Suggested research priorities*) will need to consider whether FAF imaging is intended to supplement or replace existing imaging modalities.[111] According to clinical experts (see *Chapter 1*, *Current service provision*), FAF could potentially replace fluorescein angiography for diagnosing inherited retinal dystrophies, such as retinitis pigmentosa and Stargardt disease. FAF imaging has also been proposed as a replacement for fluorescein angiography in certain monitoring applications, for example, in monitoring the progression of Best's disease,[57] verifying the results of laser therapy in retinal oedema,[112] or monitoring outcomes of macular hole surgery,[113] but evidence of whether FAF imaging can completely replace angiography in these roles is currently lacking. In the majority of retinal conditions FAF imaging is likely to be applied as an additional test, and would probably accompany optical coherence tomography and other tests, such as angiography or fundus photography, as needed.

Review of the primary evidence suggests that guidance on how to standardise FAF imaging is limited and this would need to be considered before formal recommendation of the use of FAF imaging could be made. It is unclear which guidance, if any, is being currently followed in NHS practice for classifying and interpreting FAF images. Potentially relevant methods that might serve as a starting point for further development and evaluation include those of the International Fundus Autofluorescence Classification group,[45,46] although the classification of FAF patterns is currently descriptive. Development of quantitative diagnostic criteria based on FAF patterns may help to reduce subjectivity of interpretation.[114]

Suggested research priorities

- Prospective studies that conform to the paradigm for test accuracy assessments (i.e. those that include a clearly specified population, index test, reference standard and diagnostic outcomes) would be helpful to evaluate the diagnostic accuracy of FAF imaging in the inherited retinal dystrophies, early AMD, GA and CSC.
- Prospective studies that conform to the paradigm for test accuracy assessments would be helpful to evaluate the accuracy of FAF imaging in monitoring the progression of retinal conditions and their response to therapy, alongside current best practice, in the inherited retinal dystrophies, early AMD, GA and CSC.
- Future test accuracy studies for FAF imaging should:
    - recruit participants who are representative of those likely to present for retinal screening in the NHS
    - employ all relevant components of currently used reference standards
    - clearly report the clinical information required to interpret FAF images in order to reach diagnostic and/or therapeutic decisions
    - consider carefully whether FAF imaging is appropriate as an ancillary test or as a replacement for an existing test
    - assess intergrader and intragrader agreement
    - assess other aspects of test acceptability (e.g. patient acceptability, adverse events).
- Future test accuracy studies for FAF imaging should also report clearly the duration of imaging and any resources associated with the acquisition, processing, quality assurance and interpretation of FAF images. This would assist any evaluations of the cost-effectiveness of FAF imaging that are conducted for the NHS.
- A survey or audit of the current use of FAF imaging in NHS practice would be helpful to clarify current practice and any limitations and research requirements associated with it. In particular, a survey or audit of the extent to which FAF imaging is currently used for different retinal conditions and which standards are currently employed for ensuring consistency of test results would assist prioritisation of areas where NHS resources may be needed to support the future development of FAF imaging.
- Research would be helpful to clarify which guidance, if any, is currently being followed in NHS practice for classifying and interpreting FAF images.

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Dr Geoff K Frampton (Senior Research Fellow, evidence synthesis) developed the research protocol, assessed studies for inclusion, extracted data from and critically appraised the included studies, synthesised the evidence, drafted and edited the final report and project managed the review.

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Data sharing statement

All data relevant to this technology assessment report are provided in the accompanying appendices.

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Appendix 1 Search strategy

Database, host, years searched, date of search	Literature search strategy	Results
MEDLINE(R), via Ovid, 1990 to November week 1 2014, searched 13 November 2014	1 (autofluorescen* and (fundus or fundi or fundal)).tw. (892) 2 FAF.tw. (569) 3 1 or 2 (1180) 4 topcon.tw. (407) 5 'confocal scanning laser ophthalmoscop*'.tw. (572) 6 CSLO.tw. (163) 7 optos.tw. (52) 8 spectralis.tw. (337) 9 imagenet*.tw. (79) 10 bluepeak.tw. (2) 11 'heidelberg retina angiograph'.tw. (92) 12 'heidelberg engineering'.tw. (365) 13 'AO SLO'.tw. (22) 14 'spectral domain OCT'.tw. (447) 15 optovue.tw. (77) 16 'carl zeiss meditec'.tw. (653) 17 or/4-16 (2552) 18 (fluorescen* or autofluorescen*).tw. (316,708) 19 Fluorescence/ (32,554) 20 or/18-19 (325,649) 21 17 and 20 (287) 22 3 or 21 (1287) 23 (image* or imaging).tw. (668,379) 24 (camera* or photograph* or laser* or infrared or ophthalmoscop* or instrument*).tw. (418,062) 25 Tomography, Optical Coherence/ (15,722) 26 Fluorescein Angiography/ (18,783) 27 Optical Imaging/ (1622) 28 Electroretinography/ (14,273) 29 Microscopy, Confocal/ (44,481) 30 (diagnos* or electrodiagnos*).tw. (1,620,715)	855

Database, host, years searched, date of search	Literature search strategy	Results
	31 Diagnosis, Computer-Assisted/ (20,766)	
	32 Lasers/du, is [Diagnostic Use, Instrumentation] (4259)	
	33 Image Processing, Computer-Assisted/ (100,139)	
	34 (automat* adj5 (detect* or captur* or quantif*)).tw. (10,352)	
	35 or/23-34 (2,559,128)	
	36 22 and 35 (992)	
	37 exp Retinal Diseases/ (102,626)	
	38 (retina* or retinitis or retinopath* or epiretina* or subretina* or preretina* or posterioretina* or intraretina* or chorioretinopath* or vitreoretinopath*).tw. (167,490)	
	39 (macula* or maculopath* or 'wet AMD' or 'dry AMD' or 'exud* AMD').tw. (38,891)	
	40 ((fundus or fundi or fundal) adj5 (change* or impair* or disease* or disorder* or detect* or diagnos*)).tw. (2365)	
	41 (geographical adj atroph*).tw. (11)	
	42 hyperfluorescen*.tw. (822)	
	43 (RVO or CRVO or BRVO).tw. (1256)	
	44 (cone*1 adj2 dystroph*).tw. (850)	
	45 or/37-44 (212,953)	
	46 36 and 45 (929)	
	47 (comment or editorial or letter).pt. (1,326,748)	
	48 46 not 47 (922)	
	49 limit 48 to english language (855)	

Database, host, years searched, date of search	Literature search strategy	Results
MEDLINE(R) In-Process & Other Non-Indexed Citations, via Ovid, searched from 1990 to 12 November 2014, searched on 13 November 2014	As per MEDLINE	112
EMBASE, via Ovid, searched from 1990 to 12 November 2014, searched 13 November 2014	1 (autofluorescen* and (fundus or fundi or fundal)).tw. (1043) 2 FAF.tw. (673) 3 1 or 2 (1384) 4 topcon.tw. (486) 5 'confocal scanning laser ophthalmoscop*'.tw. (582) 6 CSLO.tw. (169) 7 optos.tw. (56) 8 spectralis.tw. (479) 9 imagenet*.tw. (84) 10 bluepeak.tw. (1) 11 'heidelberg retina angiograph'.tw. (104) 12 'heidelberg engineering'.tw. (424) 13 'AO SLO'.tw. (29) 14 'spectral domain OCT'.tw. (603) 15 optovue.tw. (93) 16 'carl zeiss meditec'.tw. (695) 17 or/4-16 (3004) 18 (fluorescen* or autofluorescen*).tw. (379,356) 19 Fluorescence/ (89,241) 20 or/18-19 (393,399) 21 17 and 20 (344) 22 3 or 21 (1517) 23 (image* or imaging).tw. (906,976) 24 (camera* or photograph* or laser* or infrared or ophthalmoscop* or instrument*).tw. (556,941) 25 optical coherence tomography/ (20,952) 26 Fluorescein Angiography/ (14,527) 27 fluorescence imaging/ (6870) 28 Electroretinography/ (11,726) 29 confocal microscopy/ (40,822)	1152

Database, host, years searched, date of search	Literature search strategy	Results
	30 (diagnos* or electrodiagnos*).tw. (2,271,388)	
	31 computer assisted diagnosis/ (31,733)	
	32 Laser/ and Diagnosis/ (1798)	
	33 image processing/ (49,197)	
	34 (automat* adj5 (detect* or captur* or quantif*)).tw. (13,596)	
	35 or/23-34 (3,455,191)	
	36 22 and 35 (1154)	
	37 autofluorescence imaging/ (1207)	
	38 36 or 37 (2000)	
	39 exp Retinal Disease/ (184,028)	
	40 chorioretinopathy/ (1483)	
	41 retina* vein occlusion/ (3370)	
	42 retina macula degeneration/ or retina macula age related degeneration/ or retina maculopathy/ or retina degeneration/ or subretinal neovascularization/ (32,777)	
	43 diabetic retinopathy/ (28,376)	
	44 (retina* or retinitis or retinopath* or epiretina* or subretina* or preretina* or posterioretina* or intraretina* or chorioretinopath* or vitreoretinopath*).tw. (193,702)	
	45 (macula* or maculopath* or 'wet AMD' or 'dry AMD' or 'exud* AMD').tw. (47,694)	
	46 ((fundus or fundi or fundal) adj5 (change* or impair* or disease* or disorder* or detect* or diagnos*)).tw. (3129)	
	47 (geographical adj atroph*).tw. (11)	
	48 hyperfluorescen*.tw. (1120)	
	49 (RVO or CRVO or BRVO).tw. (1782)	
	50 (cone*1 adj2 dystroph*).tw. (951)	
	51 or/39-50 (296,820)	
	52 38 and 51 (1294)	
	53 (autofluorescence and fund* and imag*).tw. (765)	
	54 (FAF and imag*).tw. (275)	
	55 53 or 54 (776)	
	56 52 or 55 (1359)	
	57 limit 56 to (human and yr='1990 -Current') (1248)	
	58 limit 57 to english language (1152)	

Database, host, years searched, date of search	Literature search strategy	Results
SCI-E and CPCI-S, Web of Science, searched from 1990–2014, searched on 13 November 2014	# 1 (TS=(FAF and imag*)) AND LANGUAGE: (English) (233) # 2 (TS=((autofluorescen* and imag*) and (fundus or fundi or fundal))) (732) # 3 (TS=('confocal scanning laser ophthalmoscop*')) (592) # 4 (TS=(Topcon or optos or spectralis or bluepeak or 'Heidelberg retina angiograph' or 'AO SLO' or 'spectral domain OCT' or optovue)) (1404) # 5 (TS=(image* or imaging or electroretinography)) AND LANGUAGE: (English) (1,325,626) # 6 #5 AND #4 (860) # 7 (TS=(autofluorescenc* and ('fundus camera*' or 'fundus spectrophotometry' or 'confocal scan*' or 'laser ophthalmoscop*' or CsLO))) (311) # 8 (TS=(retina* or retinitis or retinopath* or epiretina* or subretina* or preretina* or posterioretina* or intraretina* or chorioretinopath* or vitreoretinopath*)) (150,838) # 9 (TS=(macula* or maculopath* or 'wet AMD' or 'dry AMD' or 'exud* AMD')) (45,128) # 10 (TS=((fundus or fundi or fundal) NEAR (change* or impair* or disease* or disorder* or detect* or diagnos*))) (3652) # 11 (TS=(geographical adj atroph*)) (80) # 12 (TS=(hyperfluorescen*)) (548) # 13 (TS=(RVO or CRVO or BRVO)) (1097) # 14 (TS=(cone* NEAR dystroph*)) (1301) # 15 #7 OR #6 OR #3 OR #2 OR #1 (2053) # 16 #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 (175,649) # 17 #16 AND #15 (1669) # 18 (TI=('fundus autofluorescence imaging')) (38) #19 #18 OR #17 (1661)	1661

Database, host, years searched, date of search	Literature search strategy	Results
The Cochrane Library, all years, searched 13 November 2014. Results found only in CDSR. Issue 11 of 12, November 2014 and CENTRAL Issue 10 of 12, October 2014 (nothing in HTA, NHS Economic Evaluation Database, DARE Issue 4 October 2014) (Also nothing unique in Cochrane Eyes and Vision group)	#1 (autofluorescen* and (fundus or fundi or fundal)) (25) #2 FAF (31) #3 #1 or #2 (44) #4 topcon (74) #5 'confocal scanning laser ophthalmoscop*' (30) #6 cSLO (8) #7 optos (6) #8 spectralis (17) #9 imagenet (13) #10 bluepeak (0) #11 'Heidelberg retina* angiograph*' (12) #12 'Heidelberg Engineering' (46) #13 'AO SLO' (0) #14 'spectral domain OCT' (16) #15 optovue (6) #16 'carl zeiss meditec' (73) #17 #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 (240) #18 (fluorescen* or autofluorescen*) (2613) #19 MeSH descriptor: [Fluorescence] this term only (185) #20 #18 or #19 (2613) #21 #17 and #20 (19) #22 #3 or #21 (53) #23 (image* or imaging) (27,896) #24 (camera* or photograph* or laser* or infrared or ophthalmoscop* or instrument*) (43,198) #25 MeSH descriptor: [Tomography, Optical Coherence] this term only (527) #26 MeSH descriptor: [Fluorescein Angiography] this term only (545) #27 MeSH descriptor: [Optical Imaging] explode all trees (593) #28 MeSH descriptor: [Electroretinography] explode all trees (140)	37 (4 CDSR, 33 CENTRAL)

Database, host, years searched, date of search	Literature search strategy	Results
	#29 MeSH descriptor: [Microscopy, Confocal] explode all trees (164) #30 (diagnos* or electrodiagnos*) (101,633) #31 MeSH descriptor: [Diagnosis, Computer-Assisted] explode all trees (1610) #32 MeSH descriptor: [Lasers] explode all trees and with qualifier(s): [Diagnostic use - DU] (150) #33 MeSH descriptor: [Image Processing, Computer-Assisted] explode all trees (2935) #34 (automat* N/5 (detect* or captur* or quantif*)) (124) #35 #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 (148,379) #36 #22 and #35 (37)	
CRD databases: DARE, HTA, NHS EED. Searched from 1990 to 12 November 2014, searched on 13 November 2014	1 ((autofluorescen* and (fundus or fundi or fundal))) FROM 1990 TO 2014 (0) 2 (FAF) FROM 1990 TO 2014 (0) 3 (#1 or #2) FROM 1990 TO 2014 (0) 4 (topcon) FROM 1990 TO 2014 (2) 5 (('confocal scanning laser ophthalmoscop*')) FROM 1990 TO 2014 (2) 6 (cslo) FROM 1990 TO 2014 (1) 7 (optos) FROM 1990 TO 2014 (0) 8 (spectralis) FROM 1990 TO 2014 (0) 9 (imagenet) FROM 1990 TO 2014 (0) 10 (bluepeak) FROM 1990 TO 2014 (0) 11 ((heidelberg retina* angiograph*)) FROM 1990 TO 2014 (0) 12 (heidelberg engineering) FROM 1990 TO 2014 (0) 13 ('AO SLO') FROM 1990 TO 2014 (0) 14 ('spectral domain') AND (OCT) FROM 1990 TO 2014 (1) 15 (optovue) FROM 1990 TO 2014 (0) 16 (carl zeiss meditec) FROM 1990 TO 2014 (1) 17 (#4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16) FROM 1990 TO 2014 (5) 18 ((fluorescen* or autofluorescen*)) FROM 1990 TO 2014 (141)	Two NHS EED (three results from HTA were all glaucoma – agreed not to download these ones; zero from DARE)

Database, host, years searched, date of search	Literature search strategy	Results
	19 MeSH DESCRIPTOR Fluorescence EXPLODE ALL TREES (16) 20 (#18 or #19) FROM 1990 TO 2014 (141) 21 (#17 and #20) FROM 1990 TO 2014 (0) 22 ((image* or imaging)) FROM 1990 TO 2014 (2889) 23 ((camera* or photograph* or laser* or infrared or ophthalmoscop* or instrument*)) FROM 1990 TO 2014 (4177) 24 MeSH DESCRIPTOR Tomography, Optical Coherence EXPLODE ALL TREES (20) 25 MeSH DESCRIPTOR Tomography, Optical EXPLODE ALL TREES (21) 26 MeSH DESCRIPTOR Fluorescein Angiography EXPLODE ALL TREES (11) 27 MeSH DESCRIPTOR Optical Imaging EXPLODE ALL TREES (29) 28 MeSH DESCRIPTOR Electroretinography EXPLODE ALL TREES (3) 29 MeSH DESCRIPTOR Microscopy, Confocal EXPLODE ALL TREES (9) 30 ((diagnos* or electrodiagnos*)) FROM 1990 TO 2014 14978 31 MeSH DESCRIPTOR Diagnosis, Computer-Assisted EXPLODE ALL TREES (114) 32 MeSH DESCRIPTOR lasers EXPLODE ALL TREES WITH QUALIFIER DU (5) 33 MeSH DESCRIPTOR Image Processing, Computer-Assisted EXPLODE ALL TREES (168) 34 ((automat* NEAR (detect* or captur* or quantif*))) FROM 1990 TO 2014 (11) 35 (#22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34) FROM 1990 TO 2014 (18,561) 36 (#4 or #5 or #6 or #14 or #16) FROM 1990 TO 2014 (5) 37 (#35 and #36) FROM 1990 TO 2014 (5)	

CDSR, Cochrane Database of Systematic Reviews; CENTRAL, Cochrane Central Register of Controlled Trials; CRD, Centre for Reviews and Dissemination; SCI-E, Science Citation Index Expanded; DARE, Database of Abstracts of Reviews of Effects; HTA, Health Technology Assessment; NHS EED, NHS Economic Evaluation Database.

Appendix 2 Study selection worksheet

Study selection worksheet for full records	**Reviewer 1:**		**Reviewer 2:**
Lead author name and Ref ID Number:			
Research type: Does the study report results of primary research with an adequate sample size (≥10 eyes per retinal condition)?	Yes ↓ next question	Unclear ↓ next question	No → EXCLUDE
Index test: Does the study report using FAF imaging for a retinal condition? Exclude tumours or secondary retinal conditions (e.g. caused by drug toxicity or other ocular conditions such as glaucoma)	Yes ↓ next question	Unclear ↓ next question	No → EXCLUDE
Reference standard:[a] Does the study report the use of one or more of: fundus fluorescein angiography, indocyanine green angiography, optical coherence tomography, fundus photography or other standard imaging test(s) for diagnosis or monitoring of a retinal condition?	Yes ↓ next question	Unclear ↓ next question	No → EXCLUDE
Diagnostic data: Does the study report sensitivity and/or specificity data for FAF imaging or data that could be used to calculate sensitivity or specificity?	Yes ↓ next question	Unclear ↓ next question	No → EXCLUDE
Final Decision	**INCLUDE**	**UNCLEAR (Discuss)**	**EXCLUDE**
Additional questions for level of evidence			
Is the study prospective, retrospective or unclear?			
Does the study report both sensitivity AND specificity data for FAF imaging?			

[a]May not be specifically referred to as a reference standard or gold standard.

Appendix 3 Critical appraisal worksheet

Critical appraisal criteria (based on Reitsma and colleagues[81] adaptation of the QUADAS Tool[93])

	Item	Description	Judgement (yes/no/unclear)
1	Was the spectrum of patients representative of the patients who will receive the test in practice?		
2	Is the reference standard likely to classify the target condition correctly?		
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?		
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?		
5	Did patients receive the same reference standard irrespective of the index test result?		
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?		
7	Were the reference standard results interpreted without knowledge of the results of the index test?		
8	Were the index test results interpreted without knowledge of the results of the reference standard?		
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?		
10	Were uninterpretable/ intermediate test results reported?		
11	Were withdrawals from the study explained?		

Appendix 4 Table of excluded studies with rationale

Note that some studies may have been excluded for more than one reason; the primary reason reported here is the first reason agreed by reviewers when following the study selection worksheet (see *Appendix 2*).

Study	Primary reason for exclusion
Ahn SJ, Ahn J, Park KH, Woo SJ. Multimodal imaging of occult macular dystrophy. *JAMA Ophthalmol* 2013;**131**:880–90	Sensitivity of FAF imaging not reported, not calculable
Aizawa S, Mitamura Y, Hagiwara A, Sugawara T, Yamamoto S. Changes of fundus autofluorescence, photoreceptor inner and outer segment junction line, and visual function in patients with retinitis pigmentosa. *Clin Exp Optom* 2010;**38**:597–604	Sensitivity of FAF imaging not reported, not calculable
Alten F, Heiduschka P, Clemens CR, Eter N. Multifocal electroretinography in eyes with reticular pseudodrusen. *Invest Ophthalmol Vis Sci* 2012;**53**:6263–70	Sensitivity of FAF imaging not reported, not calculable
Alten F, Clemens CR, Heiduschka P, Eter N. Characterisation of reticular pseudodrusen and their central target aspect in multi-spectral, confocal scanning laser ophthalmoscopy. *Graefes Arch Clin Exp Ophthalmol* 2014;**252**:715–21	Sensitivity of FAF imaging not reported, not calculable
Asao K, Gomi F, Sawa M, Nishida K. Additional anti-vascular endothelial growth factor therapy for eyes with a retinal pigment epithelial tear after the initial therapy. *Retina* 2014;**34**:512–18	Sensitivity of FAF imaging not reported, not calculable
Ayata A, Tatlipinar S, Kar T, Unal M, Ersanli D, Bilge AH. Near-infrared and short-wavelength autofluorescence imaging in central serous chorioretinopathy. *Br J Ophthalmol* 2009;**93**:79–82	Sensitivity of FAF imaging not reported, not calculable
Batioglu F, Demirel S, Ozmert E, Oguz YG, Ozyol P. Autofluorescence patterns as a predictive factor for neovascularization. *Optom Vis Sci* 2014;**91**:950–5	Sensitivity of FAF imaging not reported, not calculable
Bessho K, Gomi F, Harino S, Sawa M, Sayanagi K, Tsujikawa M, *et al.* Macular autofluorescence in eyes with cystoid macula edema, detected with 488 nm-excitation but not with 580 nm-excitation. *Graefes Arch Clin Exp Ophthalmol* 2009;**247**:729–34	Sensitivity of FAF imaging not reported, not calculable
Bessho K, Rodanant N, Bartsch DU, Cheng L, Koh HJ, Freeman WR. Effect of subthreshold infrared laser treatment for drusen regression on macular autofluorescence in patients with age-related macular degeneration. *Retina* 2005;**25**:981–8	No appropriate reference standard
Bonnet C, Querques G, Zerbib J, Oubraham H, Garavito RB, Puche N, *et al.* Hyperreflective pyramidal structures on optical coherence tomography in geographic atrophy areas. *Retina* 2014;**34**:1524–30	Sensitivity of FAF imaging not reported, not calculable
Boon CJF. SD-OCT and fundus autofluorescence imaging for differential diagnosis of macular and retinal dystrophies. *Ophthalmologica* 2013;**230**:15–16 (meeting abstract)	Sensitivity of FAF imaging not reported, not calculable
Bottoni F, Carmassi L, Cigada M, Moschini S, Bergamini F. Diagnosis of macular pseudoholes and lamellar macular holes: is optical coherence tomography the 'gold standard'? *Br J Ophthalmol* 2008;**92**:635–9	Sensitivity of FAF imaging not reported, not calculable
Bottoni F, Eandi CM, Pedenovi S, Staurenghi G. Integrated clinical evaluation of type 2A idiopathic juxtafoveolar retinal telangiectasis. *Retina* 2010;**30**:317–26	Sensitivity of FAF imaging not reported, not calculable
Brar M, Kozak I, Cheng L, Bartsch DU, Yuson R, Nigam N, *et al.* Correlation between spectral-domain optical coherence tomography and fundus autofluorescence at the margins of geographic atrophy. *Am J Ophthalmol* 2009;**148**:439–44	Sensitivity of FAF imaging not reported, not calculable
Carreno E, Portero A, Herreras JM, Lopez MI. Assesment of fundus autofluorescence in serpiginous and serpiginous-like choroidopathy. *Eye* 2012;**26**:1232–6	Sensitivity of FAF imaging not reported, not calculable
Chen Z, Song Y. Functional and structural changes after pattern scanning laser photocoagulation in diabetic retinopathy. *Doc Ophthalmol* 2013;**127**:37 (meeting abstract)	Sensitivity of FAF imaging not reported, not calculable

Study	Primary reason for exclusion
Chen FK, Patel PJ, Coffey PJ, Tufail A, Da Cruz CL. Increased fundus autofluorescence associated with outer segment shortening in macular translocation model of neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2010;**51**:4207–12	Sensitivity of FAF imaging not reported, not calculable
Chhablani JK, Narayanan R. Fundus autofluorescence patterns in type 2A idiopathic juxtafoveolar retinal telangiectasis. *Eur J Ophthalmol* 2012;**22**:398–403	Sensitivity of FAF imaging not reported, not calculable
Chia A, Tan A, Boon KL, Cheung G. Autofluorescence imaging in children with retinal pathology identified with visual electrophysiology. *Doc Ophthalmol* 2013;**127**:9 (meeting abstract)	No appropriate reference standard
Chung H, Park B, Shin HJ, Kim HC. Correlation of fundus autofluorescence with spectral-domain optical coherence tomography and vision in diabetic macular edema. *Ophthalmology* 2012;**119**:1056–65	Sensitivity of FAF imaging not reported, not calculable
Clemens CR, Alten F, Baumgart C, Heiduschka P, Eter N. Quantification of retinal pigment epithelium tear area in age-related macular degeneration. *Retina* 2014;**34**:24–31	No appropriate reference standard
Cuba J, Gomez-Ulla F. Fundus autofluorescence: applications and perspectives. *Arch Soc Esp Oftalmol* 2013;**88**:50–5	Sensitivity of FAF imaging not reported, not calculable
De Bats F, Wolff B, Mauget-Faysse M, Meunier I, Denis P, Kodjikian L. Association of reticular pseudodrusen and early onset drusen. *ISRN Ophthalmol* 2013;**2013**	Sensitivity of FAF imaging not reported, not calculable
de Laat P, Smeitink JA, Janssen MC, Keunen JE, Boon CJ. Mitochondrial retinal dystrophy associated with the m.3243A>G mutation. *Ophthalmology* 2013;**120**:2684–96	Sensitivity of FAF imaging not reported, not calculable
Deli A, Moetteli L, Ambresin A, Mantel I. Comparison of fundus autofluorescence images acquired by the confocal scanning laser ophthalmoscope (488 nm excitation) and the modified Topcon fundus camera (580 nm excitation). *Int Ophthalmol* 2013;**33**:635–43	Sensitivity of FAF imaging not reported, not calculable
Dinc UA, Tatlipinar S, Yenerel M, Gorgun E, Ciftci F. Fundus autofluorescence in acute and chronic central serous chorioretinopathy. *Clin Exp Optom* 2011;**94**:452–7	Sensitivity of FAF imaging not reported, not calculable
Dinc UA, Tatlipinar S, Gorgun E, Yenerel M. Fundus autofluorescence in optic disc drusen: comparison of confocal scanning laser ophthalmoscope and standard fundus camera. *Neuroophthalmology* 2009;**33**:318–21	Sensitivity of FAF imaging not reported, not calculable
Duncker T, Tabacaru MR, Lee W, Tsang SH, Sparrow JR, Greenstein VC. Comparison of near-infrared and short-wavelength autofluorescence in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2013;**54**:585–91	Sensitivity of FAF imaging not reported, not calculable
Duncker T, Greenberg JP, Ramachandran R, Hood DC, Smith RT, Hirose T, *et al.* Quantitative fundus autofluorescence and optical coherence tomography in Best vitelliform macular dystrophy. *Invest Ophthalmol Vis Sci* 2014;**55**:1471–82	Sensitivity of FAF imaging not reported, not calculable
Einbock W, Moessner A, Schnurrbusch UE, Holz FG, Wolf S, FAM study group. Changes in fundus autofluorescence in patients with age-related maculopathy. Correlation to visual function: a prospective study. *Graefes Arch Clin Exp Ophthalmol* 2005;**243**:300–5	Sensitivity of FAF imaging not reported, not calculable
Ergun E, Hermann B, Wirtitsch M, Unterhuber A, Ko TH, Sattmann H, *et al.* Assessment of central visual function in Stargardt's disease/fundus flavimaculatus with ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci* 2005;**46**:310–16	Sensitivity of FAF imaging not reported, not calculable
Erol MK, Ozdemir O, Coban DT, Ceran BB, Bulut M. Ranibizumab treatment for choroidal neovascularization secondary to causes other than age-related macular degeneration with good baseline visual acuity. *Semin Ophthalmol* 2014;**29**:108–13	Sensitivity of FAF imaging not reported, not calculable
Finger RP, Charbel IP, Schmitz-Valckenberg S, Holz FG, Scholl HN. Long-term effectiveness of intravitreal bevacizumab for choroidal neovascularization secondary to angioid streaks in pseudoxanthoma elasticum. *Retina* 2011;**31**:1268–78	Sensitivity of FAF imaging not reported, not calculable
Finger RP, Charbel IP, Ladewig M, Gotting C, Holz FG, Scholl HP. Fundus autofluorescence in Pseudoxanthoma elasticum. *Retina* 2009;**29**:1496–505	Sensitivity of FAF imaging not reported, not calculable
Finger RP, Charbel IP, Ladewig M, Holz FG, Scholl HP. Intravitreal bevacizumab for choroidal neovascularisation associated with pseudoxanthoma elasticum. *Br J Ophthalmol* 2008;**92**:483–7	Sensitivity of FAF imaging not reported, not calculable

Study	Primary reason for exclusion
Forte R, Querques G, Querques L, Leveziel N, Benhamou N, Souied EH. Multimodal evaluation of foveal sparing in patients with geographic atrophy due to age-related macular degeneration. *Retina* 2013;**33**:482–9	Sensitivity of FAF imaging not reported, not calculable
Forte R, Querques G, Querques L, Massamba N, Le Tien V, Souied EH. Multimodal imaging of dry age-related macular degeneration. *Acta Opthalmol* 2012;**90**:e281–7	Sensitivity of FAF imaging not reported, not calculable
Framme C, Brinkmann R, Birngruber R, Roider J. Autofluorescence imaging after selective RPE laser treatment in macular diseases and clinical outcome: a pilot study. *Br J Ophthalmol* 2002;**86**:1099–106	Sensitivity of FAF imaging not reported, not calculable
Framme C, Walter A, Gabler B, Roider J, Sachs HG, Gabel VP. Fundus autofluorescence in acute and chronic-recurrent central serous chorioretinopathy. *Acta Ophthalmol Scand* 2005;**83**:161–7	Sensitivity of FAF imaging not reported, not calculable
Framme C, Bunse A, Sofroni R, Thalhammer T, Walter A, Sachs HG, *et al.* Fundus autofluorescence before and after photodynamic therapy for choroidal neovascularization secondary to age-related macular degeneration. *Ophthalmic Surg Lasers Imaging* 2006;**37**:406–14	Sensitivity of FAF imaging not reported, not calculable
Fujiwara T, Imamura Y, Giovinazzo VJ, Spaide RF. Fundus autofluorescence and optical coherence tomographic findings in acute zonal occult outer retinopathy. *Retina* 2010;**30**:1206–16	Sensitivity of FAF imaging not reported, not calculable
Furino C, Boscia F, Cardascia N, Sborgia L, Sborgia C. Fundus autofluorescence, optical coherence tomography and visual acuity in adult-onset foveomacular dystrophy. *Ophthalmologica* 2008;**222**:240–4	Sensitivity of FAF imaging not reported, not calculable
Gajdzik-Gajdecka U, Dorecka M, Nita E, Michalska A, Miniewicz-Kurowska J, Romaniuk W. Indocyanine green angiography in chronic central serous chorioretinopathy. *Med Sci Monit* 2012;**18**:CR51–7	Sensitivity of FAF imaging not reported, not calculable
Garcia CR, Rivero ME, Bartsch DU, Ishiko S, Takamiya A, Fukui K, *et al.* Oral fluorescein angiography with the confocal scanning laser ophthalmoscope. *Ophthalmology* 1999;**106**:1114–18	No appropriate index test
Gendy MG, Fawzi AA, Wendel RT, Pieramici DJ, Miller JA, Jampol LM. Multimodal imaging in persistent placoid maculopathy. *JAMA Ophthalmol* 2014;**132**:38–49	Sensitivity of FAF imaging not reported, not calculable
Giani A, Pellegrini M, Carini E, Peroglio DA, Bottoni F, Staurenghi G. The dark atrophy with indocyanine green angiography in Stargardt disease. *Invest Ophthalmol Vis Sci* 2012;**53**:3999–4004	Sensitivity of FAF imaging not reported, not calculable
Gili P, Flores-Rodriguez P, Yanguela J, Herreros Fernàndez ML. Using autofluorescence to detect optic nerve head drusen in children. *J AAPOS* 2013;**17**:568–71	No appropriate index test
Gillies MC, Zhu M, Chew E, Barthelmes D, Hughes E, Ali H, *et al.* Familial asymptomatic macular telangiectasia type 2. *Ophthalmology* 2009;**116**:2422–9	Sensitivity of FAF imaging not reported, not calculable
Giuliari G, Hinkle DM, Foster CS. The spectrum of fundus autofluorescence findings in birdshot chorioretinopathy. *J Ophthalmol* 2009;**2009**	Sensitivity of FAF imaging not reported, not calculable
Golchet PR, Jampol LM, Mathura JR Jr, Daily MJ. Torpedo maculopathy. *Br J Ophthalmol* 2010;**94**:302–6	Inadequate sample size (< 10 eyes)
Gomes NL, Greenstein VC, Carlson JN, Tsang SH, Smith RT, Carr RE, *et al.* A comparison of fundus autofluorescence and retinal structure in patients with Stargardt disease. *Invest Ophthalmol Vis Sci* 2009;**50**:3953–9	Sensitivity of FAF imaging not reported, not calculable
Gomes NL, Corcostegui I, Fine HF, Chang S. Subfoveal pigment changes in patients with longstanding epiretinal membranes. *Am J Ophthalmol* 2009;**147**:865–8	Sensitivity of FAF imaging not reported, not calculable
Greenberg JP, Sherman J, Zweifel SA, Chen RW, Duncker T, Kohl S, *et al.* Spectral-domain optical coherence tomography staging and autofluorescence imaging in achromatopsia. *JAMA Ophthalmol* 2014;**132**:437–45	Sensitivity of FAF imaging not reported, not calculable
Greenstein VC, Santos RA, Tsang SH, Smith RT, Barile GR, Seiple W. Preferred retinal locus in macular disease: characteristics and clinical implications. *Retina* 2008;**28**:1234–40	Sensitivity of FAF imaging not reported, not calculable

Study	Primary reason for exclusion
Greenstein VC, Duncker T, Holopigian K, Carr RE, Greenberg JP, Tsang SH, *et al.* Structural and functional changes associated with normal and abnormal fundus autofluorescence in patients with retinitis pigmentosa. *Retina* 2012;**32**:349–57	Sensitivity of FAF imaging not reported, not calculable
Haas P, Esmaeelpour M, Ansari-Shahrezaei S, Drexler W, Binder S. Choroidal thickness in patients with reticular pseudodrusen using 3D 1060-nm OCT maps. *Invest Ophthalmol Vis Sci* 2014;**55**:2674–81	Sensitivity of FAF imaging not reported, not calculable
Heimes B, Lommatzsch A, Zeimer M, Gutfleisch M, Spital G, Bird AC, *et al.* Foveal RPE autofluorescence as a prognostic factor for anti-VEGF therapy in exudative AMD. *Graefes Arch Clin Exp Ophthalmol* 2008;**246**:1229–34	No appropriate reference standard
Helb HM, Charbel IP, van der Veen RL, Berendschot TT, Scholl HP, Holz FG. Abnormal macular pigment distribution in type 2 idiopathic macular telangiectasia. *Retina* 2008;**28**:808–16	Sensitivity of FAF imaging not reported, not calculable
Helb HM, Charbel IP, Fleckenstein M, Schmitz-Valckenberg S, Scholl HP, Meyer CH, *et al.* Clinical evaluation of simultaneous confocal scanning laser ophthalmoscopy imaging combined with high-resolution, spectral-domain optical coherence tomography. *Acta Opthalmol* 2010;**88**:842–9	Sensitivity of FAF imaging not reported, not calculable
Henderson RH, Mackay DS, Li Z, Moradi P, Sergouniotis P, Russell-Eggitt I, *et al.* Phenotypic variability in patients with retinal dystrophies due to mutations in CRB1. *Br J Ophthalmol* 2011;**95**:811–17	Sensitivity of FAF imaging not reported, not calculable
Heussen FM, Tan CS, Sadda SR. Prevalence of peripheral abnormalities on ultra-widefield greenlight (532 nm) autofluorescence imaging at a tertiary care center. *Invest Ophthalmol Vis Sci* 2012;**53**:6526–31	Sensitivity of FAF imaging not reported, not calculable
Heussen FMA, Fawzy NF, Joeres S, Lux A, Maaijwee K, Meurs JC, *et al.* Autologous translocation of the choroid and RPE in age-related macular degeneration: 1-year follow-up in 30 patients and recommendations for patient selection. *Eye* 2008;**22**:799-807	Sensitivity of FAF imaging not reported, not calculable
Holz FG, Bindewald-Wittich A, Fleckenstein M, Dreyhaupt J, Scholl HP, Schmitz-Valckenberg S, *et al.* Progression of geographic atrophy and impact of fundus autofluorescence patterns in age-related macular degeneration. *Am J Ophthalmol* 2007;**143**:463–72	No appropriate reference standard
Hu Z, Medioni GG, Hernandez M, Hariri A, Wu X, Sadda SR. Segmentation of the geographic atrophy in spectral-domain optical coherence tomography and fundus autofluorescence images. *Invest Ophthalmol Vis Sci* 2013;**54**:837–83	Sensitivity of FAF imaging not reported, not calculable
Imamura Y, Fujiwara T, Spaide RF. Fundus autofluorescence and visual acuity in central serous chorioretinopathy. *Ophthalmology* 2011;**118**:700–5	No appropriate reference standard
Iriyama A, Yanagi Y. Fundus autofluorescence and retinal structure as determined by spectral domain optical coherence tomography, and retinal function in retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol* 2012;**250**:333–9	Sensitivity of FAF imaging not reported, not calculable
Issa PC, Finger RP, Holz FG, Scholl HPN. Multimodal imaging including spectral domain OCT and confocal near infrared reflectance for characterization of outer retinal pathology in pseudoxanthoma elasticum. *Invest Ophthalmol Vis Sci* 2009;**50**:5913–18	Sensitivity of FAF imaging not reported, not calculable
Jarc-Vidmar M, Kraut A, Hawlina M. Fundus autofluorescence imaging in Best's vitelliform dystrophy. *Klin Monatsbl Augenheilkd* 2003;**220**:861–7	Sensitivity of FAF imaging not reported, not calculable
Jeong YJ, Hong IH, Chung JK, Kim KL, Kim HK, Park SP. Predictors for the progression of geographic atrophy in patients with age-related macular degeneration: fundus autofluorescence study with modified fundus camera. *Eye* 2014;**28**:209–18	Sensitivity of FAF imaging not reported, not calculable
Jung JJ, Khan S, Mrejen S, Gallego-Pinazo R, Cunningham ET Jr, Freund KB, *et al.* Idiopathic multifocal choroiditis with outer retinal or chorioretinal atrophy. *Retina* 2014;**34**:1439–50	Sensitivity of FAF imaging not reported, not calculable
Kaya M, Yaman A, Oner FH, Saatci AO. Autofluorescence imaging in eyes with various types of retinal artery occlusion: case report. *Turk Klin J Med Sci* 2011;**31**:1283–7	Inadequate sample size (< 10 eyes)
Kellner U, Weinitz S, Kellner S. Visualizing the onset of retinitis pigmentosa. *Doc Ophthalmol* 2010;**121**:17–18 (meeting abstract)	Sensitivity of FAF imaging not reported, not calculable

Study	Primary reason for exclusion
Kellner S, Neuhann TM, Abicht A, Wissinger B, Renner AB, Weinitz S, *et al.* Combined confocal near-infrared reflectance (815 nm) and macular spectral domain OCT identify optic atrophy in patients with bilateral unexplained visual loss. *Doc Ophthalmol* 2013;**127**:28 (meeting abstract)	Not a retinal condition
Khanifar AA, Lederer DE, Ghodasra JH, Stinnett SS, Lee JJ, Cousins SW, *et al.* Comparison of color fundus photographs and fundus autofluorescence images in measuring geographic atrophy area. *Retina* 2012;**32**:1884–91	Sensitivity of FAF imaging not reported, not calculable
Kim SK, Kim SW, Oh J, Huh K. Near-infrared and short-wavelength autofluorescence in resolved central serous chorioretinopathy: association with outer retinal layer abnormalities. *Am J Ophthalmol* 2013;**156**:157–64	Sensitivity of FAF imaging not reported, not calculable
Kim SW, Oh J, Huh K. Correlations among various functional and morphological tests in resolved central serous chorioretinopathy. *Br J Ophthalmol* 2012;**96**:350–5	Sensitivity of FAF imaging not reported, not calculable
Koizumi H, Pozzoni MC, Spaide RF. Fundus autofluorescence in birdshot chorioretinopathy. *Ophthalmology* 2008;**115**:e15–20	Sensitivity of FAF imaging not reported, not calculable
Kojima H, Otani A, Ogino K, Nakagawa S, Makiyama Y, Kurimoto M, *et al.* Outer retinal circular structures in patients with Bietti crystalline retinopathy. *Br J Ophthalmol* 2012;**96**:390–3	Sensitivity of FAF imaging not reported, not calculable
Kolomeyer AM, Baumrind BR, Szirth BC, Shahid K, Khouri AS. Fundus autofluorescence and colour fundus imaging compared during telemedicine screening in patients with diabetes. *J Telemed Telecare* 2013;**19**:209–12	Sensitivity of FAF imaging not reported, not calculable
Kolomeyer AM, Nayak NV, Szirth BC, Khouri AS. Fundus autofluorescence imaging in an ocular screening program. *Int J Telemed Appl* 2012;**2012**	Inadequate sample size (< 10 eyes)
Kramer M, Priel E. Fundus autofluorescence imaging in multifocal choroiditis: beyond the spots. *Ocul Immunol Inflamm* 2014;**22**:349–55	Inadequate sample size (< 10 eyes)
Kumar N, Mrejen S, Fung AT, Marsiglia M, Loh BK, Spaide RF. Retinal pigment epithelial cell loss assessed by fundus autofluorescence imaging in neovascular age-related macular degeneration. *Ophthalmology* 2013;**120**:334–41	Sensitivity of FAF imaging not reported, not calculable
Kurz-Levin MM, Halfyard AS, Bunce C, Bird AC, Holder GE. Clinical variations in assessment of bull's-eye maculopathy. *Arch Ophthalmol* 2002;**120**:567–75	Inadequate sample size (< 10 eyes)
Lai WW, Leung GY, Chan CW, Yeung IY, Wong D. Simultaneous spectral domain OCT and fundus autofluorescence imaging of the macula and microperimetric correspondence after successful repair of rhegmatogenous retinal detachment. *Br J Ophthalmol* 2010;**94**:311–18	Sensitivity of FAF imaging not reported, not calculable
Landa G, Rosen RB, Pilavas J, Garcia PM. Drusen characteristics revealed by spectral-domain optical coherence tomography and their corresponding fundus autofluorescence appearance in dry age-related macular degeneration. *Ophthalmic Res* 2012;**47**:81–6	Sensitivity of FAF imaging not reported, not calculable
Lee CS, Lee AY, Forooghian F, Bergstrom CS, Yan J, Yeh S. Fundus autofluorescence features in the inflammatory maculopathies. *Clin Ophthalmol* 2014;**8**:2001–12	No appropriate reference standard
Lee JE, Lim DW, Bae HY, Park HJ. Photoreceptor layer map using spectral-domain optical coherence tomography. *Optom Vis Sci* 2009;**86**:E1320–27	Sensitivity of FAF imaging not reported, not calculable
Lee JY, Lee DH, Lee JY, Yoon YH. Correlation between subfoveal choroidal thickness and the severity or progression of nonexudative age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2013;**54**:7812–18	Sensitivity of FAF imaging not reported, not calculable
Lee M, Yoon J, Ham DI. Clinical features of reticular pseudodrusen according to the fundus distribution. *Br J Ophthalmol* 2012;**96**:1222–6	Sensitivity of FAF imaging not reported, not calculable
Lee MY, Yoon J, Ham DI. Clinical characteristics of reticular pseudodrusen in Korean patients. *Am J Ophthalmol* 2012;**153**:530–5	Sensitivity of FAF imaging not reported, not calculable
Lee MY, Ham DI. Subretinal drusenoid deposits with increased autofluorescence in eyes with reticular pseudodrusen. *Retina* 2014;**34**:69–76	Sensitivity of FAF imaging not reported, not calculable
Lee TJ, Hwang JC, Chen RW, Lima LH. The role of fundus autofluorescence in late-onset retinitis pigmentosa (LORP) diagnosis. *Ophthalmic Genet* 2014;**35**:170–9	No appropriate reference standard

Study	Primary reason for exclusion
Lenassi E, Troeger E, Wilke R, Hawlina M. Correlation between macular morphology and sensitivity in patients with retinitis pigmentosa and hyperautofluorescent ring. *Invest Ophthalmol Vis Sci* 2012;**53**:47–52	Sensitivity of FAF imaging not reported, not calculable
Lenassi E, Troeger E, Wilke R, Tufail A, Hawlina M, Jeffery G, *et al.* Laser clearance of drusen deposit in patients with autosomal dominant drusen (p.Arg345Trp in EFEMP1). *Am J Ophthalmol* 2013;**155**:190–8	Sensitivity of FAF imaging not reported, not calculable
Leon PE, Saviano S, Zanei A, Pastore MR, Guaglione E, Mangogna A, *et al.* Spontaneous or secondary to intravitreal injections of anti-angiogenic agents retinal pigment epithelial tears in age-related macular degeneration. *Int J Ophthalmol* 2014;**7**:681–5	Sensitivity of FAF imaging not reported, not calculable
Levinson RD, Monnet D. Imaging in birdshot chorioretinopathy. *Int Ophthalmol Clin* 2012;**52**:191–8	Not primary research
Lima LH, Laud K, Freund KB, Yannuzzi LA, Spaide RF. Acquired vitelliform lesion associated with large drusen. *Retina* 2012;**32**:647–51	Sensitivity of FAF imaging not reported, not calculable
Lima LH, Greenberg JP, Greenstein VC, Smith RT, Sallum JM, Thirkill C, *et al.* Hyperautofluorescent ring in autoimmune retinopathy. *Retina* 2012;**32**:1385–94	Inadequate sample size (< 10 eyes)
Lima LH, Cella W, Greenstein VC, Wang NK, Busuioc M, Smith RT, *et al.* Structural assessment of hyperautofluorescent ring in patients with retinitis pigmentosa. *Retina* 2009;**29**:1025–31	Sensitivity of FAF imaging not reported, not calculable
Lima LH, Burke T, Greenstein VC, Chou CL, Cella W, Yannuzzi LA, *et al.* Progressive constriction of the hyperautofluorescent ring in retinitis pigmentosa. *Am J Ophthalmol* 2012;**153**:718–27	Sensitivity of FAF imaging not reported, not calculable
Lindner E, Weinberger A, Kirschkamp T, El-Shabrawi Y, Barounig A. Near-infrared autofluorescence and indocyanine green angiography in central serous chorioretinopathy. *Ophthalmologica* 2012;**227**:34–8	Sensitivity of FAF imaging not reported, not calculable
Liu DN, Liu Y, Meng XH, Yin ZQ. The characterization of functional disturbances in Chinese patients with Bietti's crystalline dystrophy at different fundus stages. *Graefes Arch Clin Exp Ophthalmol* 2012;**250**:191–200	Sensitivity of FAF imaging not reported, not calculable
Lois N, Owens SL, Coco R, Hopkins J, Fitzke FW, Bird AC. Fundus autofluorescence in patients with age-related macular degeneration and high risk of visual loss. *Am J Ophthalmol* 2002;**133**:341–9	Sensitivity of FAF imaging not reported, not calculable
Lois N, McBain V, Abdelkader E, Scott NW, Kumari R. Retinal pigment epithelial atrophy in patients with exudative age-related macular degeneration undergoing anti-vascular endothelial growth factor therapy. *Retina* 2013;**33**:13–22	Sensitivity of FAF imaging not reported, not calculable
Lorenz B, Wabbels B, Wegscheider E, Hamel CP, Drexler W, Preising MN. Lack of fundus autofluorescence to 488 nanometers from childhood on in patients with early-onset severe retinal dystrophy associated with mutations in RPE65. *Ophthalmology* 2004;**111**:1585–94	Sensitivity of FAF imaging not reported, not calculable
Luttrull JK, Sramek C, Palanker D, Spink CJ, Musch DC. Long-term safety, high-resolution imaging, and tissue temperature modeling of subvisible diode micropulse photocoagulation for retinovascular macular edema. *Retina* 2012;**32**:375–86	Sensitivity of FAF imaging not reported, not calculable
MacLaren RE, Uppal GS, Balaggan KS, Tufail A, Munro PMG, Milliken AB, *et al.* Autologous transplantation of the retinal pigment epithelium and choroid in the treatment of neovascular age-related macular degeneration. *Ophthalmology* 2007;**114**:561–70	Sensitivity of FAF imaging not reported, not calculable
Makiyama Y, Ooto S, Hangai M, Takayama K, Uji A, Oishi A, *et al.* Macular cone abnormalities in retinitis pigmentosa with preserved central vision using adaptive optics scanning laser ophthalmoscopy. *PLOS ONE* 2013;**8**:e79447	Sensitivity of FAF imaging not reported, not calculable
Margolis R, Mukkamala SK, Jampol LM, Spaide RF, Ober MD, Sorenson JA, *et al.* The expanded spectrum of focal choroidal excavation. *Arch Ophthalmol* 2011;**129**:1320–5	Sensitivity of FAF imaging not reported, not calculable
Marsiglia M, Boddu S, Bearelly S, Xu L, Breaux BE Jr, Freund KB, *et al.* Association between geographic atrophy progression and reticular pseudodrusen in eyes with dry age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2013;**54**:7362–9	No appropriate reference standard
Maruko I, Iida T, Ojima A, Sekiryu T. Subretinal dot-like precipitates and yellow material in central serous chorioretinopathy. *Retina* 2011;**31**:759–65	Sensitivity of FAF imaging not reported, not calculable

Study	Primary reason for exclusion
Mathew R, Papavasileiou E, Sivaprasad S. Autofluorescence and high-definition optical coherence tomography of retinal artery occlusions. *Clin Ophthalmol* 2010;**4**:1159-63	Inadequate sample size (< 10 eyes)
Matsumoto H, Kishi S, Sato T, Mukai R. Fundus autofluorescence of elongated photoreceptor outer segments in central serous chorioretinopathy. *Am J Ophthalmol* 2011;**151**:617–23.	Sensitivity of FAF imaging not reported, not calculable
Maurizio BP, Pierluigi I, Stelios K, Stefano V, Marialucia C, Ilaria Z, *et al.* Retro-mode imaging and fundus autofluorescence with scanning laser ophthalmoscope of retinal dystrophies. *BMC Ophthalmol* 2012;**12**:8	No appropriate reference standard
McBain VA, Townend J, Lois N. Fundus autofluorescence in exudative age-related macular degeneration. *Br J Ophthalmol* 2007;**91**:491–6	Sensitivity of FAF imaging not reported, not calculable
McBain VA, Kumari R, Townend J, Lois N. Geographic atrophy in retinal angiomatous proliferation. *Retina* 2011;**31**:1043–52	Sensitivity of FAF imaging not reported, not calculable
Mehta H, Davidson A, Devary S, Egan C, Hykin P, Moore A, *et al.* Autofluorescence and spectral-domain OCT findings in an atrophic maculopathy associated with pseudoxanthoma elasticum. *Clin Experiment Ophthalmol* 2012;**40**(Suppl. 1):49–50 (meeting abstract)	Sensitivity of FAF imaging not reported, not calculable
Meleth AD, Mettu P, Agron E, Chew EY, Sadda SR, Ferris FL, *et al.* Changes in retinal sensitivity in geographic atrophy progression as measured by microperimetry. *Invest Ophthalmol Vis Sci* 2011;**52**:1119–26	Sensitivity of FAF imaging not reported, not calculable
Mendis R, Lois N. Healing of the retinal pigment epithelium (RPE) imaged 'in vivo' in patients. *Clin Exp Ophthalmol* 2011;**39**(Suppl. 1):69–70 (meeting abstract)	Sensitivity of FAF imaging not reported, not calculable
Mesquida M, Llorenc V, Fontenla JR, Navarro MJ, Adan A. Use of ultra-wide-field retinal imaging in the management of active Behcet retinal vasculitis. *Retina* 2014;**34**:2121–7	Sensitivity of FAF imaging not reported, not calculable
Meyerle CB, Smith RT, Barbazetto IA, Yannuzzi LA. Autofluorescence of basal laminar drusen. *Retina* 2007;**27**:1101–6	Inadequate sample size (< 10 eyes)
Midena E, Vujosevic S, Convento E, Manfre' A, Cavarzeran F, Pilotto E. Microperimetry and fundus autofluorescence in patients with early age-related macular degeneration. *Br J Ophthalmol* 2007;**91**:1499–1503	Sensitivity of FAF imaging not reported, not calculable
Mones J, Biarnes M, Trindade F. Hyporeflective wedge-shaped band in geographic atrophy secondary to age-related macular degeneration: an underreported finding. *Ophthalmology* 2012;**119**:1412–19	Sensitivity of FAF imaging not reported, not calculable
Muqit MM, Gray JC, Marcellino GR, Henson DB, Young LB, Charles SJ, *et al.* Fundus autofluorescence and fourier-domain optical coherence tomography imaging of 10 and 20 millisecond Pascal retinal photocoagulation treatment. *Br J Ophthalmol* 2009;**93**:518–25	Sensitivity of FAF imaging not reported, not calculable
Murakami T, Akimoto M, Ooto S, Suzuki T, Ikeda H, Kawagoe N, *et al.* Association between abnormal autofluorescence and photoreceptor disorganization in retinitis pigmentosa. *Am J Ophthalmol* 2008;**145**:687–94	Sensitivity of FAF imaging not reported, not calculable
Oh J, Kim SW, Kwon SS, Oh IK, Huh K. Correlation of fundus autofluorescence gray values with vision and microperimetry in resolved central serous chorioretinopathy. *Invest Ophthalmol Vis Sci* 2012;**53**:179–84	Sensitivity of FAF imaging not reported, not calculable
Ojima A, Iida T, Sekiryu T, Maruko I, Sugano Y. Photopigments in central serous chorioretinopathy. *Am J Ophthalmol* 2011;**151**:94–152	Sensitivity of FAF imaging not reported, not calculable
Olsen TW. The Minnesota Grading System using fundus autofluorescence of eye bank eyes: a correlation to age-related macular degeneration (an AOS thesis). *Trans Am Ophthalmol Soc* 2008;**106**:383–401	Sensitivity of FAF imaging not reported, not calculable
Ooto S, Ellabban AA, Ueda-Arakawa N, Oishi A, Tamura H, Yamashiro K, *et al.* Reduction of retinal sensitivity in eyes with reticular pseudodrusen. *Am J Ophthalmol* 2013;**156**:1184–91	Sensitivity of FAF imaging not reported, not calculable
Pang CE, Shah VP, Sarraf D, Freund KB. Ultra-widefield imaging with autofluorescence and indocyanine green angiography in central serous chorioretinopathy. *Am J Ophthalmol* 2014;**158**:362–71	Sensitivity of FAF imaging not reported, not calculable

Study	Primary reason for exclusion
Pang CE, Freund KB. Ghost maculopathy: an artifact on near-infrared reflectance and multicolor imaging masquerading as chorioretinal pathology. *Am J Ophthalmol* 2014;**158**:171–8	Not a retinal condition
Park B, Kim J, Chung H, Kim HC. Correlation of fundus autofluorescence with foveal microstructures and vision in branch retinal vein occlusion. *Retina* 2014;**34**:531–8	Sensitivity of FAF imaging not reported, not calculable
Park SP, Siringo FS, Pensec N, Hong IH, Sparrow J, Barile G, *et al.* Comparison of fundus autofluorescence between fundus camera and confocal scanning laser ophthalmoscope-based systems. *Ophthalmic Surg Lasers Imaging Retina* 2013;**44**:536–43	Sensitivity of FAF imaging not reported, not calculable
Parodi MB, Iacono P, Ravalico G. Fundus autofluorescence in subfoveal choroidal neovascularisation secondary to Pathological Myopia. *Br J Ophthalmol* 2009;**93**:771–4	Sensitivity of FAF imaging not reported, not calculable
Pece A, Isola V, Holz F, Milani P, Brancato R. Autofluorescence imaging of cystoid macular edema in diabetic retinopathy. *Ophthalmologica* 2010;**224**:230–5	Sensitivity of FAF imaging not reported, not calculable
Peng Q, Dong Y, Zhao PQ. Fundus autofluorescence in exudative age-related macular degeneration. *Genet Mol Res* 2013;**12**:6140–8	Sensitivity of FAF imaging not reported, not calculable
Peng X-J, Su L-P. Characteristics of fundus autofluorescence in cystoid macular edema. *Chin Med J* 2011;**124**:253–7	Sensitivity of FAF imaging not reported, not calculable
Pichi F, Morara M, Veronese C, Nucci P, Ciardella AP. Multimodal imaging in hereditary retinal diseases. *J Ophthalmol* 2013;**2013**	Sensitivity of FAF imaging not reported, not calculable
Pilotto E, Sportiello P, Alemany-Rubio E, Vujosevic S, Segalina S, Fregona I, *et al.* Confocal scanning laser ophthalmoscope in the retromode imaging modality in exudative age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 2013;**251**:27–34	Sensitivity of FAF imaging not reported, not calculable
Pilotto E, Vujosevic S, Grgic VA, Sportiello P, Convento E, Secchi AG, *et al.* Retinal function in patients with serpiginous choroiditis: a microperimetry study. *Graefes Arch Clin Exp Ophthalmol* 2010;**248**:1331–7	Inadequate sample size (< 10 eyes)
Pilotto E, Guidolin F, Convento E, Spedicato L, Vujosevic S, Cavarzeran F, *et al.* Fundus autofluorescence and microperimetry in progressing geographic atrophy secondary to age-related macular degeneration. *Br J Ophthalmol* 2013;**97**:62–6	No appropriate reference standard
Pilotto E, Vujosevic S, Melis R, Convento E, Sportiello P, Alemany-Rubio E, *et al.* Short wavelength fundus autofluorescence versus near-infrared fundus autofluorescence, with microperimetric correspondence, in patients with geographic atrophy due to age-related macular degeneration. *Br J Ophthalmol* 2011;**95**:1140–4	No appropriate reference standard
Pryds A, Larsen M. Foveal function and thickness after verteporfin photodynamic therapy in central serous chorioretinopathy with hyperautofluorescent subretinal deposits. *Retina* 2013;**33**:128–35	Sensitivity of FAF imaging not reported, not calculable
Puche N, Querques G, Blanco-Garavito R, Zerbib J, Gherdaoui F, Tilleul J, *et al.* En face enhanced depth imaging optical coherence tomography features in adult onset foveomacular vitelliform dystrophy. *Graefes Arch Clin Exp Ophthalmol* 2014;**252**:555–62	No appropriate index test
Querques G, Zerbib J, Georges A, Massamba N, Forte R, Querques L, *et al.* Multimodal analysis of the progression of Best vitelliform macular dystrophy. *Mol Vis* 2014;**20**:575–92	Sensitivity of FAF imaging not reported, not calculable
Querques G, Leveziel N, Benhamou N, Voigt M, Soubrane G, Souied EH. Analysis of retinal flecks in fundus flavimaculatus using optical coherence tomography. *Br J Ophthalmol* 2006;**90**:1157–62	Sensitivity of FAF imaging not reported, not calculable
Querques G, Querques L, Forte R, Massamba N, Blanco R, Souied EH. Precursors of type 3 neovascularization: a multimodal imaging analysis. *Retina* 2013;**33**:1241–8	Sensitivity of FAF imaging not reported, not calculable
Querques L, Querques G, Forte R, Souied E. Microperimetric correlations of autofluorescence and optical coherence tomography imaging in dry age-related macular degeneration. *Am J Ophthalmol* 2012;**153**:1110–15	Sensitivity of FAF imaging not reported, not calculable
Querques G, Guigui B, Leveziel N, Querques L, Coscas G, Soubrane G, *et al.* Insights into pathology of cuticular drusen from integrated confocal scanning laser ophthalmoscopy imaging and corresponding spectral domain optical coherence tomography. *Graefes Arch Clin Exp Ophthalmol* 2011;**249**:1617–25	Sensitivity of FAF imaging not reported, not calculable

Study	Primary reason for exclusion
Querques G, Querques L, Martinelli D, Massamba N, Coscas G, Soubrane G, *et al.* Pathologic insights from integrated imaging of reticular pseudodrusen in age-related macular degeneration. *Retina* 2011;**31**:518–26	Sensitivity of FAF imaging not reported, not calculable
Querques G, Atmani K, Bouzitou-Mfoumou R, Leveziel N, Massamba N, Souied EH. Preferential hyperacuity perimeter in best vitelliform macular dystrophy. *Retina* 2011;**31**:959–66	Sensitivity of FAF imaging not reported, not calculable
Renner AB, Kellner U, Cropp E, Preising MN, MacDonald IM, van den Hurk JA, *et al.* Choroideremia: variability of clinical and electrophysiological characteristics and first report of a negative electroretinogram. *Ophthalmology* 2006;**113**:2066e.1–10	Sensitivity of FAF imaging not reported, not calculable
Renner AB, Kellner U, Fiebig B, Cropp E, Foerster MH, Weber BH. ERG variability in X-linked congenital retinoschisis patients with mutations in the RS1 gene and the diagnostic importance of fundus autofluorescence and OCT. *Doc Ophthalmol* 2008;**116**:97–109	Sensitivity of FAF imaging not reported, not calculable
Reznicek L, Seidensticker F, Stumpf C, Kampik A, Thurau S, Kernt M, *et al.* Systematic analysis of wide-field fundus autofluorescence (FAF) imaging in posterior uveitis. *Curr Eye Res* 2014;**39**:164–71	Sensitivity of FAF imaging not reported, not calculable
Reznicek L, Dabov S, Haritoglou C, Kampik A, Kernt M, Neubauer AS. Green-light fundus autofluorescence in diabetic macular edema. *Int J Ophthalmol* 2013;**6**:75–80	Sensitivity of FAF imaging not reported, not calculable
Reznicek L, Dabov S, Kayat B, Liegl R, Kampik A, Ulbig M, *et al.* Scanning laser 'en face' retinal imaging of epiretinal membranes. *Saudi J Ophthalmol* 2014;**28**:134–8	Sensitivity of FAF imaging not reported, not calculable
Reznicek L, Wasfy T, Stumpf C, Kampik A, Ulbig M, Neubauer AS, *et al.* Peripheral fundus autofluorescence is increased in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2012;**53**:2193–8	No appropriate reference standard
Robson AG, Lenassi E, Saihan Z, Luong VA, Fitzke FW, Holder GE, *et al.* Comparison of fundus autofluorescence with photopic and scotopic fine matrix mapping in patients with retinitis pigmentosa: 4- to 8-year follow-up. *Invest Ophthalmol Vis Sci* 2012;**53**:6187–95	Sensitivity of FAF imaging not reported, not calculable
Robson AG, Tufail A, Fitzke F, Bird AC, Moore AT, Holder GE, *et al.* Serial imaging and structure-function correlates of high-density rings of fundus autofluorescence in retinitis pigmentosa. *Retina* 2011;**31**:1670–9	Sensitivity of FAF imaging not reported, not calculable
Rofagha S, Bhisitkul RB, Boyer DS, Sadda SR, Zhang K, SEVEN-UP Study Group. Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). *Ophthalmology* 2013;**120**:2292–9	Sensitivity of FAF imaging not reported, not calculable
Roisman L, Lavinsky D, Magalhaes F, Aggio FB, Moraes N, Cardillo JA, *et al.* Fundus autofluorescence and spectral domain OCT in central serous chorioretinopathy. *J Ophthalmol* 2011;**2011**	Sensitivity of FAF imaging not reported, not calculable
Sallo FB, Rechtman E, Peto T, Stanescu-Segall D, Vogt G, Bird AC, *et al.* Functional aspects of drusen regression in age-related macular degeneration. *Br J Ophthalmol* 2009;**93**:1345–50	Sensitivity of FAF imaging not reported, not calculable
Sarks J, Arnold J, Ho I-V, Sarks S, Killingsworth M. Evolution of reticular pseudodrusen. *Br J Ophthalmol* 2011;**95**:979–85	Sensitivity of FAF imaging not reported, not calculable
Sayegh RG, Simader C, Scheschy U, Montuoro A, Kiss C, Sacu S, *et al.* A systematic comparison of spectral-domain optical coherence tomography and fundus autofluorescence in patients with geographic atrophy. *Ophthalmology* 2011;**118**:1844–51	Sensitivity of FAF imaging not reported, not calculable
Schachar IH, Zahid S, Comer GM, Stem M, Schachar AG, Saxe SJ, *et al.* Quantification of fundus autofluorescence to detect disease severity in nonexudative age-related macular degeneration. *JAMA Ophthalmol* 2013;**131**:1009–15	Sensitivity of FAF imaging not reported, not calculable
Schmitz-Valckenberg S, Fleckenstein M, Gobel AP, Sehmi K, Fitzke FW, Holz FG, *et al.* Evaluation of autofluorescence imaging with the scanning laser ophthalmoscope and the fundus camera in age-related geographic atrophy. *Am J Ophthalmol* 2008;**146**:183–92	No appropriate reference standard

Study	Primary reason for exclusion
Schmitz-Valckenberg S, Bultmann S, Dreyhaupt J, Bindewald A, Holz FG, Rohrschneider K. Fundus autofluorescence and fundus perimetry in the junctional zone of geographic atrophy in patients with age-related macular degeneration.[Erratum published in *Invest Ophthalmol Vis Sci* 2005;**46**:7]. *Invest Ophthalmol Vis Sci* 2004;**45**:4470–6	No appropriate reference standard
Schmitz-Valckenberg S, Fleckenstein M, Gobel AP, Hohman TC, Holz FG. Optical coherence tomography and autofluorescence findings in areas with geographic atrophy due to age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2011;**52**:1–6	Sensitivity of FAF imaging not reported, not calculable
Schmitz-Valckenberg S, Jorzik J, Unnebrink K, Holz FG, FAM study group. Analysis of digital scanning laser ophthalmoscopy fundus autofluorescence images of geographic atrophy in advanced age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 2002;**240**:73–8	No appropriate reference standard
Schmitz-Valckenberg S, Bindewald-Wittich A, Dolar-Szczasny J, Dreyhaupt J, Wolf S, Scholl HP, *et al.* Correlation between the area of increased autofluorescence surrounding geographic atrophy and disease progression in patients with AMD. *Invest Ophthalmol Vis Sci* 2006;**47**:2648–54	No appropriate reference standard
Schmitz-Valckenberg S, Alten F, Steinberg JS, Jaffe GJ, Fleckenstein M, Mukesh BN, *et al.* Reticular drusen associated with geographic atrophy in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2011;**52**:5009–15	No appropriate reference standard
Schutze C, Bolz M, Sayegh R, Baumann B, Pircher M, Gotzinger E, *et al.* Lesion size detection in geographic atrophy by polarization-sensitive optical coherence tomography and correlation to conventional imaging techniques. *Invest Ophthalmol Vis Sci* 2013;**54**:739–45	Sensitivity of FAF imaging not reported, not calculable
Sekiryu T, Iida T, Maruko I, Saito K, Kondo T. Infrared fundus autofluorescence and central serous chorioretinopathy. *Invest Ophthalmol Vis Sci* 2010;**51**:4956–62	Sensitivity of FAF imaging not reported, not calculable
Shah VP, Shah SA, Mrejen S, Freund KB. Subretinal hyperreflective exudation associated with neovascular age-related macular degeneration. *Retina* 2014;**34**:1281–8	Sensitivity of FAF imaging not reported, not calculable
Shen Y, Xu X, Liu K. Fundus autofluorescence characteristics in patients with diabetic macular edema. *Chin Med J* 2014;**127**:1423–8	Sensitivity of FAF imaging not reported, not calculable
Silva R, Cachulo ML, Fonseca P, Bernardes R, Nunes S, Vilhena N, *et al.* Age-related macular degeneration and risk factors for the development of choroidal neovascularisation in the fellow eye: a 3-year follow-up study. *Ophthalmologica* 2011;**226**:110–18	Sensitivity of FAF imaging not reported, not calculable
Simader C, Sayegh RG, Montuoro A, Azhary M, Koth AL, Baratsits M, *et al.* A longitudinal comparison of spectral-domain optical coherence tomography and fundus autofluorescence in geographic atrophy. *Am J Ophthalmol* 2014;**158**:557–66	Sensitivity of FAF imaging not reported, not calculable
Smith RT, Sohrab MA, Busuioc M, Barile G. Reticular macular disease. *Am J Ophthalmol* 2009;**148**:733–43	No appropriate reference standard
Spaide RF, Klancnik JM Jr. Fundus autofluorescence and central serous chorioretinopathy. *Ophthalmology* 2005;**112**:825–33	Sensitivity of FAF imaging not reported, not calculable
Suzuki M, Gomi F, Sawa M, Ueno C, Nishida K. Changes in fundus autofluorescence in polypoidal choroidal vasculopathy during 3 years of follow-up. *Graefes Arch Clin Exp Ophthalmol* 2013;**251**:2331–7	Sensitivity of FAF imaging not reported, not calculable
Tan CS, Heussen F, Sadda SR. Peripheral autofluorescence and clinical findings in neovascular and non-neovascular age-related macular degeneration. *Ophthalmology* 2013;**120**:1271–7	Sensitivity of FAF imaging not reported, not calculable
Teke MY, Elgin U, Nalcacioglu-Yuksekkaya P, Sen E, Ozdal P, Ozturk F. Comparison of autofluorescence and optical coherence tomography findings in acute and chronic central serous chorioretinopathy. *Int J Ophthalmol* 2014;**7**:350–4	Sensitivity of FAF imaging not reported, not calculable
Toju R, Iida T, Sekiryu T, Saito M, Maruko I, Kano M. Near-infrared autofluorescence in patients with idiopathic submacular choroidal neovascularization. *Am J Ophthalmol* 2012;**153**:314–19	Sensitivity of FAF imaging not reported, not calculable
Toy BC, Krishnadev N, Indaram M, Cunningham D, Cukras CA, Chew EY, *et al.* Drusen regression is associated with local changes in fundus autofluorescence in intermediate age-related macular degeneration. *Am J Ophthalmol* 2013;**56**:532–42	Sensitivity of FAF imaging not reported, not calculable

Study	Primary reason for exclusion
Tsakonas GD, Kotsolis AI, Koutsandrea C, Georgalas I, Papaconstantinou D, Ladas ID. Multiple spots of photodynamic therapy for the treatment of severe chronic central serous chorioretinopathy. *Clin Ophthalmol* 2012;**6**:1639–44	Sensitivity of FAF imaging not reported, not calculable
Vaclavik V, Vujosevic S, Dandekar SS, Bunce C, Peto T, Bird AC. Autofluorescence imaging in age-related macular degeneration complicated by choroidal neovascularization: a prospective study. *Ophthalmology* 2008;**115**:342–6	Sensitivity of FAF imaging not reported, not calculable
Vidinova CN, Gouguchkova PT, Vidinov KN. Fundus autofluorescence in dry AMD – impact on disease progression. *Klin Monatsbl Augenheilkd* 2013;**230**:1135–41	Non-English language
von Ruckmann A, Fitzke FW, Fan J, Halfyard A, Bird AC. Abnormalities of fundus autofluorescence in central serous retinopathy. *Am J Ophthalmol* 2002;**133**:780–6	Sensitivity of FAF imaging not reported, not calculable
von Ruckmann A, Fitzke FW, Bird AC. In vivo fundus auto fluorescence in macular dystrophies. *Arch Ophthalmol* 1997;**115**:609–15	Sensitivity of FAF imaging not reported, not calculable
Vujosevic S, Vaclavik V, Bird AC, Leung I, Dandekar S, Peto T. Combined grading for choroidal neovascularisation: colour, fluorescein angiography and autofluorescence images. *Graefes Arch Clin Exp Ophthalmol* 2007;**245**:1453–60	Sensitivity of FAF imaging not reported, not calculable
Vujosevic S, Bottega E, Casciano M, Pilotto E, Convento E, Midena E. Microperimetry and fundus autofluorescence in diabetic macular edema: subthreshold micropulse diode laser versus modified early treatment diabetic retinopathy study laser photocoagulation. *Retina* 2010;**30**:908–16	Sensitivity of FAF imaging not reported, not calculable
Vujosevic S, Casciano M, Pilotto E, Boccassini B, Varano M, Midena E. Diabetic macular edema: fundus autofluorescence and functional correlations. *Invest Ophthalmol Vis Sci* 2011;**52**:442–8	Sensitivity of FAF imaging not reported, not calculable
Wakabayashi T, Sawa M, Gomi F, Tsujikawa M. Correlation of fundus autofluorescence with photoreceptor morphology and functional changes in eyes with retinitis pigmentosa. *Acta Opthalmol* 2010;**88**:e177–83	Sensitivity of FAF imaging not reported, not calculable
Wang Q, Jiang L. Fundus autofluorescence imaging of acute zonal occult outer retinopathy. *Doc Ophthalmol* 2013;**127**:25 (meeting abstract)	Sensitivity of FAF imaging not reported, not calculable
Weinberger AW, Lappas A, Kirschkamp T, Mazinani BA, Huth JK, Mohammadi B, *et al.* Fundus near infrared fluorescence correlates with fundus near infrared reflectance. *Invest Ophthalmol Vis Sci* 2006;**47**:3098–108	Sensitivity of FAF imaging not reported, not calculable
Wolf-Schnurrbusch UE, Enzmann V, Brinkmann CK, Wolf S. Morphologic changes in patients with geographic atrophy assessed with a novel spectral OCT-SLO combination. *Invest Ophthalmol Vis Sci* 2008;**49**:3095–9	Sensitivity of FAF imaging not reported, not calculable
Wong WT, Forooghian F, Majumdar Z, Bonner RF, Cunningham D, Chew EY. Fundus autofluorescence in type 2 idiopathic macular telangiectasia: correlation with optical coherence tomography and microperimetry. *Am J Ophthalmol* 2009;**148**:573–83	Sensitivity of FAF imaging not reported, not calculable
Yoshitake S, Murakami T, Horii T, Uji A, Ogino K, Unoki N, *et al.* Qualitative and quantitative characteristics of near-infrared autofluorescence in diabetic macular edema. *Ophthalmology* 2014;**121**:1036–44	Sensitivity of FAF imaging not reported, not calculable

Appendix 5 Data extraction tables

Appendix 1 Data extraction tables

Study 1 of 8 – Cachulo and colleagues

Reference and design	Diagnostic tests	Participants	Outcome measures
Condition being diagnosed / detected: Choroidal neovascularisation (CNV) in exudative AMD **First author:** Cachulo[99] **Publication year:** 2011 **Country:** Portugal **Study design:** Prospective observational longitudinal 2 year study **Number of centres:** One **Funding:** Not reported **Competing interests:** Not reported 1 author appears to be employed by Pfizer Inc.	**Index test:** Fundus autofluorescence (FAF): acquired with confocal scanning laser ophthalmoscopy (cSLO) HRA II (Heidelberg Retina Angiograph) Excitation 488nm; barrier filter beginning at 500nm. Each FAF image was compiled from at least 17 single scans in movie mode and automatically aligned and averaged. **Reference standard:** Fluorescein angiography (FA): acquired using the HRA II (Heidelberg Retina Angiograph) scanning laser ophthalmoscope **Comparator:** 1) Colour fundus photography 2) Fluorescein angiography 3) Indocyanine green angiography 4) Optical coherence tomography 5) Retinal angiography (retinal leakage analysis – RLA – measuring retinal fluorescein leakage from the blood stream into the vitreous using cSLO)	**Number of participants:** 62 (52 included in analysis) **Number of eyes:** 52 **Sample attrition/dropout:** 52 participants completed the 2 year follow-up, dropout was due to death (4 patients), withdrawal of informed consent (4 patients), hospitalisation (1 patient), loss to follow-up (1 patient treated in another country) **Selection of participants:** Patients with neovascular AMD in one eye and early AMD in the fellow eye (study eye) at risk for development of CNV. Not reported whether patients selected consecutively **Inclusion criteria for study entry:** 1) Older than 50 years 2) Any race and either sex 3) Clinical diagnosis of wet AMD in one eye (non-study eye) 4) Presence of the following characteristics in the	**Primary outcome of study:** Presence of conversion from early AMD to wet AMD: sensitivity and specificity (repeated imaging assessments at 6-monthly intervals for 2 years or until CNV presence was confirmed in the study eye) **Other relevant outcomes:** None **Diagnostic threshold:** FAF (observed from results, but not stated in methods): patchy pattern; reticular pattern; speckled pattern; focal increased pattern; lacelike pattern FA: not reported **Recruitment dates:** Not reported

		study eye: a) 5 or more intermediate soft drusen >63µm or 1 large soft druse >125µm, and/or confluent drusen within 3,000 µm of the foveal centre b) With or without pigmentary changes **Exclusion criteria for study entry:** 1) Current or past medical condition that would preclude scheduled visits or completion of the study 2) Current or past history of ophthalmic disease in the study eye (other than AMD), that would likely compromise the visual acuity of the study eye 3) Clinical signs of myopic retinopathy or refractive power of >8 diopters or funduscopic evidence of degenerative myopia 4) Past history of intraocular surgery within 60 days prior to enrolling in the study 5) Evidence of past or present CNV in the study eye	

Participant characteristics	
Sex, m:f (%male)	26:26 (50)
Age, years, mean (SD)	76 (6), range 56-92

Results – FAF versus FA

Calculations are based on number of eyes (single eyes of 52 subjects)	**Population with disease on FA reference standard**		**Population without disease on FA reference standard**		**Total**
FAF imaging positive	15	a	23	c	38
FAF imaging negative	2	b	12	d	14
Total	17		35		52

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	88.24 %	63.52 to 98.20
Clinical specificity d / (c + d)	34.29 %	19.15 to 52.21
PPV a / (a + c)	39.47 %	24.05 to 56.61
NPV d / (b + d)	85.71 %	57.16 to 97.80
Positive likelihood ratio [sensitivity/(100-specificity)]	1.34	1.00 to 1.80
Negative likelihood ratio [(100-sensitivity)/specificity]	0.34	0.09 to 1.36
Diagnostic odds ratio (a x d)/(b x c)	3.91	0.77 to 20.02

Comments: Calculations do not agree with values reported in paper. Reported values for FAF are: sensitivity 93%, specificity 37%, positive predictive value 57% and negative predictive value 93%. This may be because of different ways that the reviewer and authors categorised the 2 eyes in FAF in which the pattern of autofluorecence could not be determined because of poor quality images.

Interpretability and acceptability of test	
Numbers excluded from analysis due to poor image quality	2/52 (3.85%)
Inter-observer agreement	Not reported
Intra-observer agreement	Not reported
Test acceptability (patients / clinicians)	Not reported
Adverse events	Not reported

AMD: age-related macular degeneration; CNV: choroidal neovascularisation; cSLO: confocal scanning laser ophthalmoscopy; FA: fluorescein angiography; FAF: fundus autofluorescence; NPV: negative predictive value; PPV: positive predictive value.

Cachulo and colleagues[99] critical appraisal criteria (based on Reitsma et al.[81] adaptation of the QUADAS Tool[93])

	Item	Description	Judgement
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	Study is prospective but unclear if it involved consecutive patients. Participants had confirmed CNV in one eye, so may be an atypical case-mix	No
2	Is the reference standard likely to classify the target condition correctly?	FA is described as the gold standard for assuming conversion from early AMD to wet AMD	Yes
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	It is reported that each patient underwent study assessments at baseline and every six months for two years. However, no detail is given about the time between tests	Unclear
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	Of 62 patients enrolled in the study, 10 dropped out, due to death (n=4), withdrawal of consent (n=4), hospitalisation (n=1) and loss to follow up (n=1). It is confirmed in the results section that 52/52 of the remaining patients underwent the fluorescein angiography test	Yes
5	Did patients receive the same reference standard irrespective of the index test result?	The results confirm that 52/52 patients underwent the fluorescein angiography test	Yes
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	Yes	Yes
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Not reported	Unclear
8	Were the index test results interpreted without knowledge of the results of the reference standard?	Not reported	Unclear
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Not reported	Unclear
10	Were uninterpretable or intermediate test results reported?	It is stated that in 2 eyes the pattern of autofluorescence could not be determined because of poor quality images	Yes
11	Were withdrawals from the study explained?	Yes	Yes

Study 2 of 8 – Dinc and colleagues

Reference and design	Diagnostic tests	Participants	Outcome measures
Condition being diagnosed / detected: Cystoid macular oedema (CMO) (secondary to diabetic retinopathy, retinal vein occlusions, uveitis, cataract surgery, epiretinal membrane or age-related macular degeneration) **First author:** Dinc[83] **Publication year:** 2010 **Country:** Turkey (not stated explicitly) **Study design:** Patients were selected from a FAF database (no further details given); informed consent was obtained from all patients, suggesting the study was prospective **Number of centres:** Not explicitly reported but appears to be single centre **Funding:** No information provided **Competing interests:** No information provided	**Index test:** Fundus autofluorescence (FAF) acquired with confocal scanning laser ophthalmoscopy (cSLO) (Heidelberg Retinal Angiograph 2, Heidelberg Engineering, Germany). View mode 30°; pupil dilated to a diameter ≥ 6 mm. Excitation 488nm; barrier filter 500nm. Stated that a mean of 9 frames was obtained. **Reference standard:** Fluorescein angiography (FA). Method not reported except that in the late phase of FA, pathognomonic leakage of fluorescein at the fovea in a petaloid configuration with feathery margins was considered as CMO. **Comparator:** Optical coherence tomography (OCT) (type not reported)	**Number of participants:** 55 **Number of eyes:** 67 **Sample attrition/dropout:** None (results reported for all eyes) **Selection of participants:** Stated only that the patients diagnosed with CMO were selected from a FAF database (no criteria specified) **Inclusion criteria for study entry:** Patients with CMO secondary to diabetic retinopathy, retinal vein occlusions, uveitis, cataract surgery, epiretinal membrane or age-related macular degeneration **Exclusion criteria for study entry:** Eyes with significant media opacity, cataract, poor FAF images, or having subfoveal serous retinal detachment on OCT	**Primary outcome of study:** Detection of CMO by FAF and FA **Other relevant outcomes:** Central macular thickness assessed by OCT (data not extracted here) **Diagnostic threshold:** Not explicitly stated but implied to be increased autofluorescence in a round or oval fashion at the fovea (example image given for reference) **Recruitment dates:** Unclear. Stated that patients were selected from the FAF database between January 2008 and June 2009

Participant characteristics	
Sex, m:f (%male)	28:27 (51)
Age, years, mean (SD)	62.1 (14.4)
Origin of CMO (n= no. of eyes)	Diabetic retinopathy, n=36 Branch retinal vein occlusion, n=13 Macular epiretinal membrane, n=5 Age-related macular degeneration, n=5 Uveitis, n=4 Cataract extraction, n=3 Central retinal vein occlusion, n=1

Results – FAF compared against FA

Calculations are based on the numbers of eyes (both eyes of 12 subjects and single eyes of 43 subjects)	**Population with CMO on FA**		**Population without CMO on FA**		**Total**
FAF imaging positive	64	a	2	c	66
FAF imaging negative	1	b	0	d	1
Total	65		2		67

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	98.46%	91.69 to 99.74
Clinical specificity d / (c + d)	0.00%	0.00 to 80.71
PPV a / (a + c)	96.97%	89.46 to 99.54
NPV d / (b + d)	0.00%	0.00 to 83.45
Positive likelihood ratio [sensitivity/(100-specificity)]	0.98	0.96 to 1.01
Negative likelihood ratio [(100-sensitivity)/specificity]	Not calculable	
Diagnostic odds ratio (a x d)/(b x c)	8.60	0.28 to 268.48
Comments: Diagnostic outcomes are not reported in paper – calculated by reviewer		

Interpretability and acceptability of test	
Numbers excluded from analysis due to poor image quality	None – results are reported for all 67 study eyes
Inter-observer agreement	Not reported
Intra-observer agreement	Not reported
Test acceptability (patients / clinicians)	Not reported
Adverse events	Not reported

CMO: cystoid macular oedema; cSLO: confocal scanning laser ophthalmoscopy; FA: fluorescein angiography; FAF: fundus autofluorescence; NPV: negative predictive value; OCT: optical coherence tomography; PPV: positive predictive value

Dinc and colleagues[83] critical appraisal criteria (based on Reitsma et al.[81] adaptation of the QUADAS Tool[93])

	Item	Description	Judgement
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	Unclear if study is prospective, but it involved consecutive patients. CMO was secondary to a range of conditions, and patients with CMO and serous retinal detachment were excluded, so is an atypical case-mix	No
2	Is the reference standard likely to classify the target condition correctly?	FA is described as the gold standard for detecting CMO	Yes
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Order, but not timing, of tests specified	Unclear
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	Whole sample	Yes
5	Did patients receive the same reference standard irrespective of the index test result?	Yes	Yes
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	Separate tests applied at different times	Yes
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Stated that the data on FA and FAF images were evaluated by as single clinician; masking not reported	Unclear
8	Were the index test results interpreted without knowledge of the results of the reference standard?	FAF images were obtained prior to FA images	Yes
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Not reported	Unclear
10	Were uninterpretable or intermediate test results reported?	All eyes included in analysis but not stated whether image quality was an inclusion or exclusion criterion	No
11	Were withdrawals from the study explained?	Results data reported for all eyes – implies no withdrawals	Not applicable

Study 3 of 8 – Hogg and colleagues

Reference and design	Diagnostic tests	Participants	Outcome measures
Condition being diagnosed / detected: Reticular pseudodrusen (RPD) in age-related macular degeneration (AMD) **First author:** Hogg[96] **Publication Year:** 2014 **Country:** Italy, Portugal, UK (Northern Ireland) **Study design:** Prospective cohort study **Number of centres:** 3 **Funding:** Educational grant from Pfizer Inc. **Competing interests:** Authors declared financial support or consultancies from Pfizer, Heidelberg Engineering, Zeiss Meditec, Novartis, Allergan, Zeiss, Alcon, Bayer, and THEA	**Index test:** fundus autofluorescence (FAF) acquired using scanning laser ophthalmoscopy (SLO): Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany). Excitation not stated; barrier filter not stated. Settings: Field of view 30° centred on the macula; automatic image brightness (also called gain); high-speed mode; movie duration 30 seconds; average of 15 frames (Spectralis mean function); and tomography settings 7mm for Z-scan images **Reference standard:** (1) Reference standard relevant to the current review: Colour fundus photography (CFP): Stereopair colour images acquired using a Topcon 50X fundus camera. No further details given. (2) Reference standard according to the primary study: Presence or absence of RPD on >1 of 5 modalities: CFP, red-free photography (RF), Infrared photography (IR), fundus auto-fluorescence (FAF), and optical coherence tomography (OCT) CFP: details as above IR: acquired using same equipment as index test and same settings	**Number of participants:** 105 **Number of eyes:** 105 **Sample attrition/dropout:** Not reported, but appears to have excluded 12 eyes with poor image quality (n=93 after exclusion) **Selection of participants:** Patients attending retina clinics at each study site who had a diagnosis of neovascular AMD in 1 eye were approached and invited to take part. Neovascular AMD not defined in the publication **Inclusion criteria for study entry:** Men and women older than 50 years with a confirmed diagnosis of neovascular AMD in 1 eye; study eye (fellow eye) free of any features of late AMD (i.e., no neovascularization or geographic atrophy) with a visual acuity of 20/40 or better; sufficiently clear ocular media and adequate pupillary dilatation to permit good-quality fundus imaging of the study eye; and	**Primary outcome of study:** Presence of RPD **Other relevant outcomes:** Between-grader repeatability (κ statistics) for each imaging method **Diagnostic threshold:** Definitions of RPD: FAF: "clusters of ill-defined hypo-autofluorescent lesions interspersed against a background of mildly increased AF occurring in a regular and well-defined array." CFP: yellow interlacing networks ranging from 125 to 250 µm in width or lesions that occurred in regular, well-defined domains. IR and RF: "clusters of ill-defined hypo-reflective lesions interspersed against a background of mild hyper-reflectance." OCT: discrete accumulations of material anterior to the RPE often occurring as sharp

	RF: acquired using same equipment as index test and same settings OCT acquired using same equipment as index test. Centred on the macula, using evenly spaced lines in the scan area: 30° (horizontal) x 15° (vertical) area; number of sections set to 37; mean function used with 5 scans per line; high-speed acquisition mode Note: SD-OCT implied but not stated	willing and able to comply with scheduled visits, laboratory tests, and other trial procedures. **Exclusion criteria for study entry:** Evidence of a neovascular lesion on FA in the study eye; any other feature of neovascular AMD (eg. subretinal or intraretinal fibrosis within the macular region, RPE tear); significant media opacities, cataracts, lens opacification requiring cataract surgery within 2 year follow-up; other retinal disease eg. pathologic myopia (spherical equivalent of -8 diopters or more or axial length of 25 mm or more), ocular istoplasmosis syndrome, angioid streaks, choroidal rupture, multifocal choroiditis; ocular progressive disease, eg. glaucoma or diabetic retinopathy in the study eye; medical condition that would interfere with the patient's ability to complete the trial; concurrent enrolment in any other observational or interventional clinical study; treatment with an ocular or systemic investigational agent in the past 60 days for medical	peaks visible within the layers corresponding to the outer regions of the photoreceptors **Recruitment dates:** Not reported

		condition; or known serious allergies to the dye used in FA or ICGA.	

Participant characteristics	
Sex, m:f (%male)	53:52 (50)
Age, years, mean (SD)	75.6 (7.5), range 52-93
Visual acuity in patients with vs. without drusen 1) Distance visual acuity (letters), mean (SD) 2) Near visual acuity (logarithm of the minimum angle of resolution), mean (SD) 3) Low luminescence visual acuity (SKILL score), mean (SD)	 1) 83 (6) vs. 81 (6) 2) 0.3 (0.1) vs. 0.2 (0.1) 3) 38 (12) vs. 33 (9)

Results – (1) FAF versus Spectralis OCT

Calculations are based on numbers of eyes (single eyes of 93 subjects)	**Population with disease on Spectral OCT**		**Population without disease on Spectral OCT**		**Total**
FAF imaging positive	29	a	9	c	38
FAF imaging negative	4	b	48	d	52
Total	33		57		90

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	87.88 %	71.78 to 96.52
Clinical specificity d / (c + d)	84.21 %	72.13 to 92.30
PPV a / (a + c)	76.32 %	59.75 to 88.53
NPV d / (b + d)	92.31 %	81.44 to 97.82
Positive likelihood ratio [sensitivity/(100-specificity)]	5.57	3.02 to 10.27
Negative likelihood ratio [(100-sensitivity)/specificity]	0.14	0.06 to 0.36
Diagnostic odds ratio (a x d)/(b x c)* *0.5 added to each number to avoid division by zero	38.67	10.92 to 136.97
Interpretability and acceptability of test – see table below		

Results – (2) FAF versus CFP

Calculations are based on numbers of eyes (single eyes of 93 subjects)	**Population with disease on CFP**		**Population without disease on CFP**		**Total**
FAF imaging positive	15	a	26	c	41
FAF imaging negative	0	b	52	d	52
Total	15		78		93

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	100.00 %	78.03 to 100.00
Clinical specificity d / (c + d)	66.67 %	55.08 to 76.94
PPV a / (a + c)	36.59 %	22.13 to 53.06
NPV d / (b + d)	100.00 %	93.08 to 100.00
Positive likelihood ratio [sensitivity/(100-specificity)]	3.00	2.19 to 4.11
Negative likelihood ratio [(100-sensitivity)/specificity]	0.00	Not calculable
Diagnostic odds ratio (a x d)/(b x c)* *0.5 added to each number to avoid division by zero	61.42	3.54 to 1066.71

Comments: CFP is the usual method for diagnosing RPD but was not the reference standard in the primary study. Diagnostic outcomes for this comparison were not reported in the paper but have been calculated by reviewers from data in Table 4 in the paper.

Interpretability and acceptability of test – see table below

Results – (3) FAF versus >1 imaging modality

Calculations are based on numbers of eyes (single eyes of 93 subjects)	**Population with disease on >1 imaging modality**		**Population without disease on >1 imaging modality**		**Total**
FAF imaging positive	41	a	0	c	41
FAF imaging negative	2	b	50	d	52
Total	43		50		93

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	95.35 %	84.16 to 99.30
Clinical specificity d / (c + d)	100.00 %	92.82 to 100.00
PPV a / (a + c)	100.00 %	91.31 to 100.00
NPV d / (b + d)	96.15 %	86.76 to 99.42
Positive likelihood ratio [sensitivity/(100-specificity)]	Not calculable	
Negative likelihood ratio [(100-sensitivity)/specificity]	0.05	0.01 to 0.18
Diagnostic odds ratio (a x d)/(b x c)	1676.60	78.30 to 35903.35

Comments: The diagnostic odds ratio was not reported in the paper. The calculation of specificity differs as the paper reported specificity to be 98%.

Interpretability and acceptability of test	
Numbers excluded from analysis due to poor image quality	Not reported. Appears to have excluded 12 eyes that were ungradable for RPD: Instead of 105 eyes, results are presented for 93 eyes comparing FAF with >1 imaging modality; 93 eyes comparing FAF with fundus photography; and 90 eyes comparing FAF with OCT. However, the numbers that were ungradable on each imaging modality are not specified.
Inter-observer agreement (only for the UK [Belfast] site, n=35), kappa statistics	Colour photography, 0.72 (P<0.001); IR, 0.87 (P<0.001); RF, 0.53 (P = 0.002); FAF, 0.94 (P<0.001); OCT, 0.86 (P<0.001); ICGA, 0.93 (P<0.001); RPD on 1 or more imaging method, 1.0.
Intra-observer agreement	Not reported
Test acceptability (patients / clinicians)	Not reported
Adverse events	Not reported

AF: autofluorescence; AMD: age-related macular degeneration; CFP: colour fundus photography; cSLO: confocal scanning laser ophthalmoscopy; FA: fluorescein angiography; FAF: fundus autofluorescence; ICGA: indocyanine green angiography; IR: infrared photography; NPV: negative predictive value; OCT: optical coherence tomography; PPV: positive predictive value; RF: red-free photography; RPD: reticular pseudodrusen; RPE: retinal pigment epithelium; SD-OCT: spectral-domain optical coherence tomography

Hogg and colleagues[96] critical appraisal criteria (based on Reitsma et al.[81] adaptation of the QUADAS Tool[93])

	Item	Description	Judgement
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	Study is prospective, and involved consecutive patients. Patients had neovascular AMD in only one eye, and no signs of AMD or other eye conditions in the other eye, so this is an atypical case-mix	No
2	Is the reference standard likely to classify the target condition correctly?	Study reference standard is positive result on ≥1 of 5 modalities (CFP, RFP, IRP, FAF, OCT), but CFP is the standard approach in clinical practice, with SD-OCT and FA also useful for detecting RPD	Unclear
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Not reported	Unclear
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	Multiple imaging methods were used, but it is unclear whether all participants received all tests but data was excluded, or whether some participants did not receive all tests. Attrition is not reported	Unclear
5	Did patients receive the same reference standard irrespective of the index test result?	No, the combination of the ≥1 test modalities making up the reference standard varied between patients.	No
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	The index test was one of the tests contributing to a diagnosis	No
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Not reported	Unclear
8	Were the index test results interpreted without knowledge of the results of the reference standard?	Not reported	Unclear
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Not reported	Unclear
10	Were uninterpretable or intermediate test results reported?	12 subjects were ungradable for RPD and these appear to have been excluded from analysis – but the number of ungradable images on the index test is not reported	No
11	Were withdrawals from the study explained?	No	No

Study 4 of 8 – McBain and colleagues

Reference and design	Diagnostic tests	Participants	Outcome measures
Condition being diagnosed/detected: Cystoid macular oedema (CMO) **First author:** McBain[100] **Publication Year:** 2008 **Country:** UK **Study design:** Retrospective, consecutive, observational case series **Number of centres:** 1 **Funding:** Not stated **Competing interests:** Stated none	**Index test:** FAF imaging using cSLO. This was obtained using Heidelberg retina angiograph which consisted of a solid-state argon blue excitation laser (488nm) and barrier filter (500nm). 30 degree field-of-view mode was used for the images. Sequential images were obtained and 20 frames were selected and averaged to assess the distribution of FAF. **Reference standard:** Fluorescein angiography (FA) Digital stereo images obtained using Topcon-Imagenet system **Comparator:** None Time period between tests: within 2 weeks of each other; there was a minimum gap of 4 days washout if FAF was obtained following FA	**Number of participants:** 34 **Number of eyes**: 34 **Sample attrition/dropout:** 106 consecutive patients with clinically suspected CMO had FAF imaging, of which 34 patients were eligible for inclusion and 62 were excluded.* **Selection of participants:** Consecutive patients with clinically suspected CMO were selected from FAF imaging database of the Ophthalmology Department. **Inclusion criteria for study entry:** CMO secondary to cataract extraction, inherited retinopathies, inflammatory eye disease or idiopathic cases, where both FAF and FA were obtained to confirm diagnosis. One eye per person included, left eye chosen in bilateral cases. Patients were eligible if FAF was performed within 2 weeks of FA **Exclusion criteria for study entry:** No additional criteria cited.	**Primary outcome of study:** Diagnostic accuracy (sensitivity and specificity) **Other relevant outcomes:** Interpretability and acceptability of test; adverse events **Diagnostic threshold:** FAF: CMO considered present whenever there were round or oval areas of fundus autofluorescence at the fovea with a fundus auto-fluorescence signal similar to back-ground levels. FAF signal is usually reduced at the fovea compared with background, due to blockage of the signal by the luteal pigment. FA: CMO was considered present whenever leakage of fluorescein dye was observed in a petaloid pattern around the fovea in the late phase of the angiogram (recirculation phase or later) **Recruitment dates:** February 2004 - May 2007*

*The numbers do not add up to 106 but 96. There is a discrepancy in reporting the total numbers in the abstract (which reports 96) vs the text (reporting 106). There is also a discrepancy in recruitment dates in the abstract (reported as between Aug 2004 and June 2006) vs the text (Feb 2004 and May 2007). It appears that the main text has been updated but the abstract has not, and that 10 exclusions have not been accounted for.

Participant characteristics	
Sex, m:f (%male:female)	20:14 (59)
Age, years, mean (SD)	59 (range 17-89)
CMO secondary to inflammatory disease, n (%)	17/34 (50)
CMO following cataract surgery, n (%)	11/34 (32)
CMO associated with inherited retinal dystrophies, n (%)	3/34 (9)
CMO idiopathic, n (%)	4/34 (12)

Results – FAF versus FA

Calculations are based on numbers of eyes (= number of patients as only one eye per patient was included)	**Population with disease on FA**		**Population without disease on FA**		**Total**
FAF imaging positive	17	a	4	c	21
FAF imaging negative	4	b	9	d	13
Total	21		13		34

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	80.95	58.08 to 94.44
Clinical specificity d / (c + d)	69.23	38.61 to 90.72
PPV a / (a + c)	80.95	58.08 to 94.44
NPV d / (b + d)	69.23	38.61 to 90.72
Positive likelihood ratio [sensitivity/(100-specicifcity)]	2.63	1.13 to 6.10
Negative likelihood ratio [(100-sensitivity)/specificity]	0.28	0.11 to 0.71
Diagnostic odds ratio (a x d)/(b x c)	9.56	1.92 to 47.57

Comments: Calculations agree with values reported in paper except for values for PPV and NPV, which are switched in the paper.

Interpretability and acceptability of test	
Poor FAF images related to media opacities (cataract), n (%)	9/96 (9.4%)
Inter-observer agreement	Not reported
Intra-observer agreement	Not reported
Test acceptability (patients / clinicians)	Not reported
Adverse events	No side effects were observed related to FAF or AF images during the study period.
The percentage has been calculated by reviewers using the denominator 96 rather than 106, as the reasons for 10 exclusions appear to have been omitted from the paper (see above).	

CMO: cystoid macular oedema; FA: fluorescein angiography; FAF: fundus autofluorescence; NPV: negative predictive value; PPV: positive predictive value

McBain and colleagues[100] critical appraisal criteria (based on Reitsma et al.[81] adaptation of the QUADAS Tool[93])

	Item	**Description**	**Judgement**
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	Study is retrospective, but involved consecutive patients. Patients had CMO secondary to specific conditions, so this may be an atypical case-mix	No
2	Is the reference standard likely to classify the target condition correctly?	FA is described as used routinely for diagnosis of CMO	Yes
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Within two weeks of each other, with a minimum gap of 4 days if FAF followed AF.	Yes
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	All included patients received both tests.	Yes
5	Did patients receive the same reference standard irrespective of the index test result?	FA was used in all analysed patients	Yes
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	FAF was not part of reference standard	Yes
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Evaluation of results was done in a masked fashion by a single observer; images evaluated independently from one another.	Unclear
8	Were the index test results interpreted without knowledge of the results of the reference standard?	As above	Unclear
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	States no information was provided to the observer with regards to the patient.	Unclear
10	Were uninterpretable or intermediate test results reported?	Reports number of patients excluded due to poor FAF images (9/96)	Yes
11	Were withdrawals from the study explained?	62/96 patients excluded: 23 no AF, 14 had more than two weeks	Yes

		between tests, 2 had FAF less than four days after FA, 9 had poor AF images related to media opacities (cataract), 14 had CMO related to other diseases, e.g. precious branch vein occlusion, diabetic retinopathy or AMD. However, there is a discrepancy between the recruitment dates and numbers recruited between the abstract and main text, which suggests that there are 10/106 exclusions which are not accounted for.	

Study 5 of 8 – Smith and colleagues

Reference and design	Diagnostic tests	Participants	Outcome measures
Condition being diagnosed / detected: Reticular pseudodrusen (RPD) in age-related macular degeneration (AMD) **First author:** Smith[101] **Publication year:** 2006 **Country:** UK and USA **Study design:** Retrospective case series (2 distinct case series combined) **Number of centres:** Not reported **Funding:** New York Community Trust (lead author) and unrestricted funds from Research to Prevent Blindness **Competing interests:** Stated none	**Index test:** Fundus autofluorescence (FAF) imaging. No details of method reported; introduction suggests probably confocal scanning laser ophthalmoscopy (cSLO) **Reference standard:** Colour fundus photography (CFP). Colour photographs were studied both in their original state and as highly contrast-enhanced versions, to facilitate RPD identification. No further details of method reported. **Comparator:** None reported	**Number of participants:** 138 **Number of eyes:** 221 (166 eyes of 83 patients with early AMD or GA, without evidence of choroidal neovascularisation (CNV)) and 55 unaffected eyes of 55 patients with unilateral CNV) **Sample attrition/dropout:** None (retrospective database selection) **Selection of participants:** From two databases: an AMD study database at the UK Institute of Ophthalmology; and a database of patients imaged at Columbia Eye University, USA. Not reported whether patients were selected consecutively. **Inclusion criteria for study entry:** Not explicitly reported. Stated only that the eyes had either: bilateral soft drusen ± pigment abnormalities, but no evidence of CNV; or they had unilateral CNV. **Exclusion criteria for study entry:** Eyes that did not receive both FAF imaging and colour fundus photography.	**Primary outcome of study:** The fraction and relative probability of focally increased autofluorescence corresponding spatially with drusen and pigment as identified by fundus colour photography; and the presence or absence of reticular FAF and RPD **Other relevant outcomes:** None reported **Diagnostic threshold:** FAF: Reticular pattern of autofluorescence (hypofluorescent lesions) CFP: Image segmentation method reported, but morphological criteria for diagnosing RPD on CFP not reported **Recruitment dates:** Not reported

Participant characteristics				
Sex, m:f (%male)	Not reported			
Age, years, mean (SD)	Not reported			
Other key characteristics	None reported			

Results – FAF imaging

Calculations are based on numbers of eyes (both eyes of 83 subjects and single eyes of 55 subjects)	**Population with RPD on colour fundus photography**		**Population without RPD on colour fundus photography**		**Total**
FAF imaging positive	28	a	4	c	32
FAF imaging negative	2	b	187	d	189
Total	30		191		221

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	93.33%	77.89 to 98.99
Clinical specificity d / (c + d)	97.91%	94.72 to 99.41
PPV a / (a + c)	87.50%	70.99 to 96.41
NPV d / (b + d)	98.94%	96.22 to 99.84
Positive likelihood ratio [sensitivity/(100-specificity)]	44.57	16.82 to 118.08
Negative likelihood ratio [(100-sensitivity)/specificity]	0.07	0.02 to 0.26
Diagnostic odds ratio (a x d)/(b x c)	654.50	114.50 to 3741.07

Comments: Sensitivity calculation agrees with statement in the paper that "AF imaging was over 90% sensitive" (no other diagnostic results were reported in the paper).
Sensitivity and specificity are also calculable for a sub-group of patients based on unaffected fellow eyes of those with unilateral CNV ("CNV-R" group). However, subgroup is defined by auto-fluorescence pattern (reticular AF and / or RPD) and does not include all patients with unilateral CNV. Therefore data have not been extracted here.

Interpretability and acceptability of test	
Numbers excluded from analysis due to poor image quality	None. However, reported that for one patient with RPD only this was "perhaps due to marginal scan quality" and another patient had "bilateral RPD and an AF image in the left eye that could not be graded for reticular AF". Unclear whether these were the only poor-quality images present.
Inter-observer agreement	Not reported
Intra-observer agreement	Not reported
Test acceptability (patients / clinicians)	Not reported
Adverse events	Not reported

AF: autofluorescence; AMD: age-related macular degeneration; CFP: colour fundus photography; CNV: choroidal neovascularisation; cSLO: scanning laser ophthalmoscopy; FAF: fundus autofluorescence; GA: geographic atrophy; NPV: negative predictive value; PPV: positive predictive value; RPD: reticular pseudodrusen

Smith and colleagues[101] critical appraisal criteria (based on Reitsma et al.[81] adaptation of the QUADAS Tool[93])

	Item	Description	Judgement
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	Study is retrospective but unclear if it involved consecutive patients. Two separate cohorts of eyes were combined: patients from the UK without evidence of CNV and unaffected eyes of patients from the USA who had unilateral CNV, so is an atypical case mix	No
2	Is the reference standard likely to classify the target condition correctly?	Although not stated explicitly, CFP is a standard approach for detecting RPD	Yes
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Not reported	Unclear
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	Whole sample – appears to have been purposively selected to ensure patients had received both tests	Yes
5	Did patients receive the same reference standard irrespective of the index test result?	Yes	Yes
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	Yes	Yes
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Not reported	Unclear
8	Were the index test results interpreted without knowledge of the results of the reference standard?	Not reported	Unclear
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Not reported	Unclear
10	Were uninterpretable or intermediate test results reported?	No. Unclear whether patient selection purposively excluded those with uninterpretable or indeterminate test results. Some poor-quality images may have influenced results classification (see 'Interpretability and acceptability of test' above)	No
11	Were withdrawals from the study explained?	No withdrawals	Not applicable

Study 6 of 8 – Ueda-Arakawa and colleagues

Reference and design	Diagnostic tests	Participants	Outcome measures
Condition being diagnosed / detected: Reticular pseudodrusen (RPD) in age-related macular degeneration (AMD) **First author:** Ueda-Arakawa[97] **Publication year:** 2013 **Country:** Japan **Study design:** Retrospective case series **Number of centres:** One **Funding:** Not stated **Competing interests:** Stated none	**Index test:** (1) Fundus auto-fluorescence (FAF): acquired using confocal scanning laser ophthal-moscope (cSLO) (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). Excitation 488nm; barrier filter beginning at 500nm. (2) Near-infrared fundus autofluorescence (NIR-FAF): acquired with cSLO (same equipment as FAF), in the indocyanine green angiography mode (excitation: 790nm; detection 800nm). Each FAF or NIR-FAF image was compiled from an average of 15 to 20 scans by the cSLO software. **Reference standard:** At least 2 of 7 imaging modalities (in any combination) positive for RPD: (1) Contrast-enhanced colour fundus photo-graphy (CFP): 30°–40° field acquired digitally using Topcon TRC NW6S non-mydriatic retinal camera (Topcon, Tokyo, Japan). Blue channel examined using Image J software (Nat-ional Institutes of Health, Bethesda, MD, USA). (NB: paper notes that this has been the traditional method for detecting RPD).	**Number of participants:** 114 **Number of eyes:** 220 **Sample attrition/dropout:** 8/228 eyes excluded, due to phthisis bulbi (n=2) or poor image quality in ≥3 imaging modalities (n=6). Further excluded due to poor image quality: FAF imaging: 3/220; NIR-FAF imaging: 84/220. **Selection of participants:** Consecutive patients with AMD who first visited ophthal-mology department during recruitment dates **Inclusion criteria for study entry:** Early AMD, neovascular AMD or geographic atrophy in at least one eye. Early AMD defined as presence of soft drusen (≥ 63 µm) or areas of hyper- or hypopigmentation in the RPE. Geographic atropy defined on colour fundus photography as a sharply delineated area (≥ 175 µm) ie hypopigmentation, depigmentation or apparent absence of RPE in which choroidal vessels were clearly visible.	**Primary outcome of study:** Not stated. Paper focuses on sensitivity and specificity of each imaging modality at detecting RPD. **Other relevant outcomes:** Inter-grader agreement rates for detecting RPD in each imaging modality. **Diagnostic threshold:** RPD diagnosed if reticular patterns showed on at least two of the follow-ing: blue-channel CFP, IR, FAF, NIR-FAF, CBR, IA, or SD-OCT. Characterisation of reticular lesions: FAF and NIR-FAF: A group of ill-defined, hypo-fluorescent lesions against a back-ground of mildly elevated AF. Blue-channel CFP and CBR: light interlacing networks 125-250µm wide. IR: groups of hypo-reflectant lesions against a back-ground of mild hyper-reflectance with anomalous characteristics.

	(2) Infrared reflectance (IR): acquired using cSLO (same equipment as the index test). Light stimulus 820nm. (3) FAF imaging (i.e., an index test – see above). (4) NIR-FAF imaging (i.e., an index test – see above). (5) Confocal blue reflectance (CBR): acquired with cSLO (same equipment as the index test). Light stimulus 488nm; field of view 30° x 30°, centred on the macula. (6) Late-phase indocyanine green angiography (ICGA): acquired with cSLO (same equipment as the index test). Excitation: 790nm; detection 800nm. (7) Spectral domain optical coherence tomography (SD-OCT): conducted using Spectralis HRA+OCT (Heidelberg Engineering). Horizontal and vertical line scans through the fovea centre obtained at a 30° angle, followed by serial horizontal scans with an examination field size ranging from 30° x 10° to 30° x 25. At each location of interest on the retina, 50 images were averaged to reduce speckle noise.	Neovascular AMD defined as neovascularisation detected using FA or indocyanine angiography. Only images of eligible quality were analysed and only eyes with eligible image quality in at least five imaging modalities were included. **Exclusion criteria for study entry:** People aged < 50 years, eyes with high myopia, eyes with other macular abnormalities.	ICGA: A distinctive grouping of hypo-fluorescent dots present in the late angiogram phases. SD-OCT: ≥5 hyper-reflective mounds or triangular lesions above the RPE in ≥1 B-scan. **Recruitment dates:** January 2010 – November 2010

Participant characteristics	
Sex, m:f (%male)	79:35 (69)
Age, years, mean (SD)	73.8 (9.4), range 52-92
Visual acuity (logarithm of the minimum angle of resolution), mean (SD)	0.396 (0.512), range 0.01-1.5

Results

FAF versus ≥2 (of 7) imaging modalities

Calculations are based on numbers of eyes, including both eyes of each subject	**Population with disease on ≥2 imaging modalities**	**Population without disease on ≥2 imaging modalities**	**Total**
FAF imaging positive	32 a	9 c	41
FAF imaging negative	5 b	171 d	176
Total	37	180	217

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	86.49%	71.21 to 95.41
Clinical specificity d / (c + d)	95.00%	90.72 to 97.68
PPV a / (a + c)	78.05%	62.38 to 89.42
NPV d / (b + d)	97.16%	93.49 to 99.06
Positive likelihood ratio [sensitivity/(100-specificity)]	17.30	9.04 to 33.11
Negative likelihood ratio [(100-sensitivity)/specificity]	0.14	0.06 to 0.32
Diagnostic odds ratio (a x d)/(b x c)	121.60	38.25 to 386.57

Comments: Calculations agree with values reported in paper (except diagnostic odds ratio not reported). Paper also reports (in Supplementary Table 2) that the sensitivity of FAF imaging is 86.5% when the field size is limited to the same imaging area as SD-OCT, i.e. 30^{o} x 10^{o} – but sample sizes (n/N) for this calculation (32/37) are not explained.

Note that CFP is the test usually considered as a reference standard for diagnosing RPD. Although diagnostic outcomes for a comparison of FAF versus CFP are given in supplementary Table 1 of the paper, these relate only to a subset of 37 eyes that had a reticular pattern on ≥2 imaging modalities, therefore these data have not been extracted.

Interpretability and acceptability of test	
Number of eyes excluded from analysis due to poor image quality	3/220 (1.4%)
Inter-observer agreement (grading reticular pattern)	89.3%; kappa = 0.700
Intra-observer agreement	Not reported
Test acceptability (patients / clinicians)	Not reported
Adverse events	Not reported

NIR-FAF versus ≥2 (of 7) imaging modalities

Calculations are based on numbers of eyes, including both eyes of each subject	**Population with disease on ≥2 imaging modalities**	**Population without disease on ≥2 imaging modalities**	**Total**
NIR-FAF imaging positive	9 a	5 c	14
NIR-FAF imaging negative	19 b	103 d	122
Total	28	108	136

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	32.14%	15.91% to 52.35%
Clinical specificity d / (c + d)	95.37%	89.52% to 98.46%
PPV a / (a + c)	64.29%	35.18% to 87.11%
NPV d / (b + d)	84.43%	76.75% to 90.35%
Positive likelihood ratio [sensitivity/(100-specificity)]	6.94	2.53 to 19.08
Negative likelihood ratio [(100-sensitivity)/specificity]	0.71	0.55 to 0.92
Diagnostic odds ratio (a x d)/(b x c)	9.76	2.95 to 32.33

Comments: Calculations agree with values reported in paper (except diagnostic odds ratio not reported). Paper also reports (in Supplementary Table 2) that the sensitivity of NIR-FAF imaging is 28.6% when the field size is limited to the same imaging area as SD-OCT, i.e. 30^{o} x 10^{o} – but sample sizes (n/N) for this calculation (8/28) are not explained.

Interpretability and acceptability of test	
Number of eyes excluded from analysis due to poor image quality	64/220 (29%)
Inter-observer agreement (grading reticular pattern)	84.2%; kappa = 0.563
Intra-observer agreement	Not reported
Test acceptability (patients / clinicians)	Not reported
Adverse events	Not reported

Number of eyes with good image quality – results for all imaging tests	
FAF	217/220 (99%)
NIR-FAF	136/220 (62%)
Blue-channel CFP	220/220 (100%)
IRR	220/220 (100%)
ICGA	220/220 (100%)
SD-OCT	220/220 (100%)
CBR	204/220 (93%)

AMD: age-related macular degeneration; CBR: confocal blue reflectance; CFP: colour fundus photography; CNV: choroidal neovascularisation; cSLO: confocal scanning laser ophthalmoscopy; FAF: fundus autofluorescence; GA: geographic atrophy; ICGA: indocyanine green angiography; IRR: infrared reflectance; NIR-FAF: near-infrared fundus autofluorescence; NPV: negative predictive value; PPV: positive predictive value; RPD: reticular pseudodrusen; RPE: retinal pigment epithelium; SD-OCT: spectral-domain optical coherence tomography

Ueda-Arakawa and colleagues[97] critical appraisal criteria (based on Reitsma et al.[81] adaptation of the QUADAS Tool[93])

	Item	Description	Judgement
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	Study is retrospective but involved consecutive patients. Patients are Japanese, with newly diagnosed AMD, and patients with comorbidities excluded (including, among others, certain types of CNV and central serous chorioretinopathy), so is an atypical case-mix	No
2	Is the reference standard likely to classify the target condition correctly?	Paper describes CFP as traditional test for detecting RPD. Study reference standard is positive result on ≥2 of 7 modalities, but individual eyes were diagnosed using different combinations of modalities	Unclear
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Not reported	Unclear
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	Multiple imaging methods used in all patients but the diagnostic tests differed between patients	Yes
5	Did patients receive the same reference standard irrespective of the index test result?	No, the combination of the ≥2 test modalities making up the reference standard varied between patients	No
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	The index test(s) could have been one (or both) of the two tests contributing to a diagnosis	No
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Not reported	Unclear
8	Were the index test results interpreted without knowledge of the results of the reference standard?	Not reported	Unclear
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Not reported	Unclear
10	Were uninterpretable or intermediate test results reported?	Stated that 3/220 eyes for FAF (1%), 84/220 eyes for NIR-FAF (38%) and 16 eyes for CBR (7%) did not have good quality images and were excluded	Yes
11	Were withdrawals from the study explained?	Yes – phthisis bulbi or poor image quality	Yes

Study 7 of 8 – Vujosevic and colleagues

Reference and design	Diagnostic tests	Participants	Outcome measures
Condition being diagnosed / detected: diabetic macular oedema (DMO) **First author:** Vujosevic[98] **Publication year:** 2012 **Country:** Italy **Study design:** Cross-sectional study. Probably prospective (not explicitly stated but all patients provided consent) **Number of centres:** Not reported (>1 clinic implied) **Funding:** None received **Competing interests:** No information provided	**Index test:** Fundus autofluorescence (FAF) acquired with confocal scanning laser ophthalmoscopy (cSLO) (Heidelberg Retinal Angiograph, HRA 2; Heidelberg Engineering, Heidelberg, Germany). Solid-pumped laser; excitation 488nm; emission detected above 500nm using barrier filter. FAF signal amplified by calculating a mean of 15 aligned images after correction of eye movements using image analysis software. **Reference standard:** Retromode scanning laser ophthalmoscopy (RM-SLO): images taken with F-10 SLO (Nidek Co, Gamagori, Japan), which uses 4 wavelengths: blue, green, red and infrared. Infrared laser was set at 790nm. F-10 contains 8 apertures (five confocal and 3 with a central stop) and five stops. To obtain a RM-SLO image, a central stop and a laterally oriented oval-shaped opening was used, from both right and left sides. **Comparators:** Time domain OCT (TD-OCT) Fluorescein angiography (FA)	**Number of participants:** 137 **Number of eyes:** 263 **Sample attrition/dropout:** Not reported explicitly but none evident **Selection of participants:** Recruited consecutively from unspecified diabetic retinopathy (DR) clinics **Inclusion criteria for study entry:** Type 1 or 2 diabetes mellitus; any stage of untreated or treated DR; and having TD-OCT, FAF, FA and RM-SLO performed on the same day **Exclusion criteria for study entry:** Significant media opacities	**Primary outcome of study:** Inter-method agreement in identifying different patterns of DMO **Other relevant outcomes:** Sensitivity and specificity for detecting DMO **Diagnostic threshold:** Identification of DMO: FAF: Not reported (stated only that images were graded for different foveal patterns [normal, single spot increased and multiple spots increased] and presence⁄absence of decreased⁄increased auto-fluorescence in the macula) TD-OCT: central retinal thickness > 230 µm (measured in the central foveal zone) FA: Not reported (stated only that late-phase FA images of the macula were graded for the presence of fluorescein leakage and pattern of leakage [cystoid and non-cystoid]). RM-SLO: Not reported (stated only that images were graded for presence/absence of

			DMO) For all methods, 2 masked retinal specialists trained in imaging grading independently graded all images on a 17-inch monitor dedicated to DR screening. In case of disagreement, a 3rd specialist adjudicated. **Recruitment dates:** March to August 2009

Participant characteristics	
Sex, m:f (%male)	87:50 (64)
Age, years, mean (SD)	Type I diabetes: 48.8 (11.5), range 28-64 Type II diabetes: 66.6 (8.1), range 41-85 Overall : Not reported (numbers with diabetes do not account for all patients – see below)
With Type I or II diabetes, N(%)	Type I: 12 (8.8) [reported as 10.1% in the paper] Type II: 107 (78.1) [reported as 89.9% in the paper] Not reported: 18 (13.1)
Duration of diabetes, years, mean (SD)	Type I: 28.8 (11.9), range 5-51 Type II: 15.4 (8.8), range 1-39
Central macular thickness, mean (SD), µm	323.4 (125.2), range 154.0-884.0

Results - FAF versus RM-SLO

Calculations based on numbers of eyes (both eyes of 126 subjects and single eyes of 11 subjects)	**Population with DMO on RM-SLO**		**Population without DMO on RM-SLO**		**Total**
FAF imaging positive	195	a	8	c	203
FAF imaging negative	16	b	44	d	60
Total	211		52		263

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	92.42%	87.98 to 95.60
Clinical specificity d / (c + d)	84.62%	71.91 to 93.10
PPV a / (a + c)	96.06%	92.38 to 98.28
NPV d / (b + d)	73.33%	60.34 to 83.92
Positive likelihood ratio [sensitivity/(100-specificity)]	6.01	3.17 to 11.38
Negative likelihood ratio [(100-sensitivity)/specificity]	0.09	0.06 to 0.15
Diagnostic odds ratio (a x d)/(b x c)	67.03	26.99 to 166.45

Comments: Data reported in the paper are for RM-SLO compared against a FAF reference; recalculated by reviewers to give sensitivity and specificity of FAF compared against a RM-SLO reference.

Paper states (in the Discussion) that OCT is the 'new gold standard' for diagnosing DMO. However, a diagnostic accuracy comparison of FAF versus TD-OCT is not possible from the reported data (only the diagnostic accuracy of RM-SLO versus TD-OCT, FA and FAF are reported and calculable – not extracted here).

Interpretability and acceptability of test	
Numbers excluded from analysis due to poor image quality	Not reported
Inter-observer agreement	Not reported
Intra-observer agreement	Not reported
Test acceptability (patients / clinicians)	Not reported
Adverse events	Not reported

cSLO: confocal scanning ophthalmoscopy; DMO: diabetic macular oedema; DR: diabetic retinopathy; FA: fluorescein angiography; FAF: fundus autofluorescence; NPV: negative predictive value; OCT: optical coherence tomography; PPV: positive predictive value; RM-SLO: retromode scanning laser ophthalmoscopy; TD-OCT: time domain optical coherence tomography

Vujosevic and colleagues[98] critical appraisal criteria (based on Reitsma et al.[81] adaptation of the QUADAS Tool[93])

	Item	Description	Judgement
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	Unclear if study is prospective, but it involved consecutive patients. Patients had any stage of untreated or treated diabetic retinopathy so this may be a typical case-mix	Unclear
2	Is the reference standard likely to classify the target condition correctly?	Paper describes OCT (i.e. not RM-SLO) as the 'new gold standard' for classifying DMO	Unclear
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Same day	Yes
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	Whole sample	Yes
5	Did patients receive the same reference standard irrespective of the index test result?	Yes	Yes
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	Yes	Yes
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Order of tests not reported but stated that images were independently graded in a masked fashion	Yes
8	Were the index test results interpreted without knowledge of the results of the reference standard?	Order of tests not reported but stated that images were independently graded in a masked fashion	Yes
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Not reported	Unclear
10	Were uninterpretable or intermediate test results reported?	Not reported	No
11	Were withdrawals from the study explained?	No withdrawals evident	Not applicable

Study 8 of 8 – Waldstein and colleagues

Reference and design	Diagnostic tests	Participants	Outcome measures
Condition being diagnosed / detected: Diabetic macular oedema (DMO) **First author:** Waldstein[84] **Publication year:** 2012 **Country:** Not stated, appears to be UK **Study design:** Retrospective cross-sectional. **Number of centres:** One **Funding:** 2 authors received Marie Curie Intra European Fellowship; Worshipful Company of Barbers-Waitangi Foundation Fellowship; and funding from the University of Auckland. **Competing interests:** Stated none	**Index tests:** FAF imaging using cSLO (modified HRA classic, Heidelberg Engineering, Heidelberg, Germany) with an external source of a solid-state laser emitting at 488nm at a 30˚ field of view; and FAF imaging using cSLO with an argon-ion laser emitting at 514nm at a 30˚ field of view; mean of 16 images. **Reference standard:** SD-OCT (Heidelberg Engineering, software version 1.6.4.0). Each B-scan consisted of 512 A-scans and was averaged nine times using the ART mode. A 20˚ x 20˚ scan pattern using 25 sections with an inter-scan distance of 240µm was recorded. **Comparator:** Macular Pigment Optical Density (MPOD) imaging (sequential use of both 488nm and 514nm FAF allowed calculation of macular pigment optical density (MPOD) maps that topographically illustrate the relative distribution of macular pigment)	**Number of participants:**71 **Number of eyes:** 125 **Sample attrition/dropout:** Not reported explicitly; but all included eyes were analysed. **Selection of participants:** Patients who underwent OCT and two-wavelength FAF imaging in the diabetic retinopathy clinic of a university hospital were selected consecutively. **Inclusion criteria for study entry:** The presence of diabetic retinopathy with or without DMO; clear ocular media that allow recording of high-quality FAF images; availability of both two-wavelength FAF and OCT imaging within a 2-week period. **Exclusion criteria for study entry:** Presence of any ocular comorbidity affecting the macula, such as retinal vein occlusion or age-related macular degeneration.	**Primary outcome of study:** Comparison of sensitivity and specificity of FAF and MPOD for detection of DMO **Other relevant outcomes:** Inter-grader variability. (Cohen's kappa was used to estimate inter-grader agreement) **Diagnostic threshold:** Diagnosis of DMO was based on DMO visibility which was compared across the technologies using the following scoring system: -no DMO visible -DMO suspected -DMO clearly visible FAF: Relatively bright, single or multiple, round or oval areas that are mostly bordered by darker rims. OCT: Intraretinal cysts (no details given) **Recruitment dates:** Between May 2009 and November 2010

Participant characteristics	
Sex, m:f (%male)	46:25 (65%)
Age, years, mean (SD)	63 (15)

Results: FAF (488nm) versus OCT

Calculations are based on no. of eyes (single eyes from 17 subjects and both eyes from 54 subjects)	**Eyes with signs of DMO on SD-OCT**		**Eyes without signs of DMO on SD-OCT**		**Total**
FAF imaging positive	54	a	6	c	60
FAF imaging negative	13	b	52	d	65
Total	67		58		125

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	80.60	69.11 to 89.24
Clinical specificity d / (c + d)	89.66	78.82 to 96.08
PPV a / (a + c)	90.00	79.48 to 96.22
NPV d / (b + d)	80.00	68.23 to 88.89
Positive likelihood ratio [sensitivity/(100-specicifcity)]	7.79	3.62 to 16.77
Negative likelihood ratio [(100-sensitivity)/specificity]	0.22	0.13 to 0.36
Diagnostic odds ratio (a x d)/(b x c)	36.00	12.73 to 101.81

Comments: Diagnostic values are calculated by the reviewer from the reported sensitivity and specificity. The calculations agree with the results reported in the paper.

Sensitivity and specificity are reported also for MPOD based on combining FAF488 nm and 514nm images. MPOD sensitivity and specificity were very similar to those of FAF 488nm alone (data not extracted here).

Results: FAF (514nm) versus OCT

Calculations are based on numbers of eyes (single eyes from 17 subjects and both eyes from 54 subjects)	**Eyes with signs of DMO on SD-OCT**		**Eyes without signs of DMO on SD-OCT**		**Total**
FAF imaging positive	37	a	3	c	40
FAF imaging negative	30	b	55	d	85
Total	67		58		125

Diagnosis		**95% CI**
Clinical sensitivity a / (a + b)	55.22	42.58 to 67.39
Clinical specificity d / (c + d)	94.83	85.60 to 98.86
PPV a / (a + c)	92.50	79.59 to 98.34
NPV d / (b + d)	64.71	53.59 to 74.77
Positive likelihood ratio [sensitivity/(100-specificity)]	10.68	3.47 to 32.82
Negative likelihood ratio [(100-sensitivity)/specificity]	0.47	0.36 to 0.62
Diagnostic odds ratio (a x d)/(b x c)	22.61	6.43 to 79.54

Comments: Diagnostic values are calculated by the reviewer from the reported sensitivity and specificity. The calculations agree with the results reported in the paper.

Distinct patterns of DMO on OCT (no. of eyes, %):	
Predominantly foveal intraretinal cysts	51 (76)
Predominantly extrafoveal intraretinal cysts	5 (7)
Diffuse, small intraretinal cysts	11 (16)
Sensitivity for detecting Foveal cysts compared to OCT imaging	
FAF (488nm)	90.0%
FAF (514nm)	20.0%
MPOD	96.0%
Sensitivity for detecting Extrafoveal or diffuse cysts compared to OCT imaging	
FAF (488nm)	60.8%
FAF (514nm)	70.0%
MPOD	45.5%
MPOD vs OCT	
Clinical sensitivity	80.6%
Clinical specificity	91.4%

FAF: Fundus Autofluorescence; NPV: negative predictive value; PPV: positive predictive value; MPOD: Macular Pigment Optical Density; SD-OCT: Spectral Domain Optical Coherence Tomography; cSLO: Confocal Scanning Laser Ophthalmoscope; DMO: Diabetic Macular Oedema

Interpretability and acceptability of test	
Numbers excluded from analysis due to poor image quality	Not reported
Intra-observer agreement	Not reported
Test acceptability (patients/clinicians)	Not reported
Adverse events	Not reported
Inter-observer agreement (Cohen's kappa)	
FAF (488nm)	0.84
FAF (514nm)	0.63
MPOD	0.79

Waldstein and colleagues[84] critical appraisal criteria (based on Reitsma et al.[81] adaptation of the QUADAS Tool[93])

	Item	Description	Judgement
1	Was the spectrum of patients representative of the patients who will receive the test in practice?	Study is retrospective, but involved consecutive patients. Patients had diabetic retinopathy with or without DMO, with no macular comorbidities, so this may be an atypical case-mix	No
2	Is the reference standard likely to classify the target condition correctly?	Paper describes OCT as clinical standard for the non-invasive diagnosis of DMO	Yes
3	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Both two-wavelength FAF and OCT imaging were available within a 2 week period.	Yes
4	Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	Patients were required to have both tests for inclusion	Yes
5	Did patients receive the same reference standard irrespective of the index test result?	Yes	Yes
6	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	Yes	Yes
7	Were the reference standard results interpreted without knowledge of the results of the index test?	Same grader who assessed OCT scans was one of the FAF graders. No masking reported.	Unclear
8	Were the index test results interpreted without knowledge of the results of the reference standard?	All FAF and MPOD were evaluated by two independent graders who were blinded to the patient but not to the mode of imaging. No further details provided.	Unclear
9	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Not reported	Unclear
10	Were uninterpretable or intermediate test results reported?	No	No
11	Were withdrawals from the study explained?	All included eyes were analysed	Not applicable

EME
HS&DR
HTA
PGfAR
PHR

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