ACADEMIC UNIT OF PRIMARY HEALTH CARE
Canynge Hall, 39 Whatley Road, Bristol.

DUTY: The Diagnosis of Urinary Tract Infections in Young Children Study.

STUDY PROTOCOL

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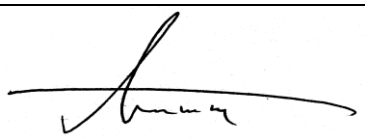
Alastair Hay	Chief Investigator		**15/01/10**

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i) Glossary of Abbreviations:

CCU	**Clean Catch Urine**
CED	**Children's Emergency Department**
CFU	**Colony Forming Unit**
CRF	**Case Report Form**
CSO	**Clinical Studies Officer**
CVU	**Clean Voided mid-stream Urine**
PCO	**Primary Care Organisation**
RN	**Research Nurse**
UTI	**Urinary Tract Infection**
WIC	**Walk-in Centre**

ii) Definitions:

Positive Culture	A growth of a single microorganism of $>10^5$ cfu/ml of urine following culture.
Borderline culture/borderline result	A growth of a single organism of between 10^4 and 10^5 cfu/ml, or a mixed growth (>1 organism) of 10^5 cfu/ml of urine following culture.
Contamination	Presence of microorganisms which are unlikely to represent urinary tract pathogens.
Research Site	One of the four partner organisations (Bristol, Cardiff, London, Southampton), at which the Principal Study Investigators are based.
Primary Care Sites	Any primary care centres (General Practices, WICs, CEDs, Out of Hours GP Cooperatives or Polyclinics) at which children will be recruited.

iii)Keywords: Urinary Tract Infection, children, primary care, point-of-care-test, dipstick test, near-patient testing.

1 Study Summary

1.1 Study Schema

Overall study schema

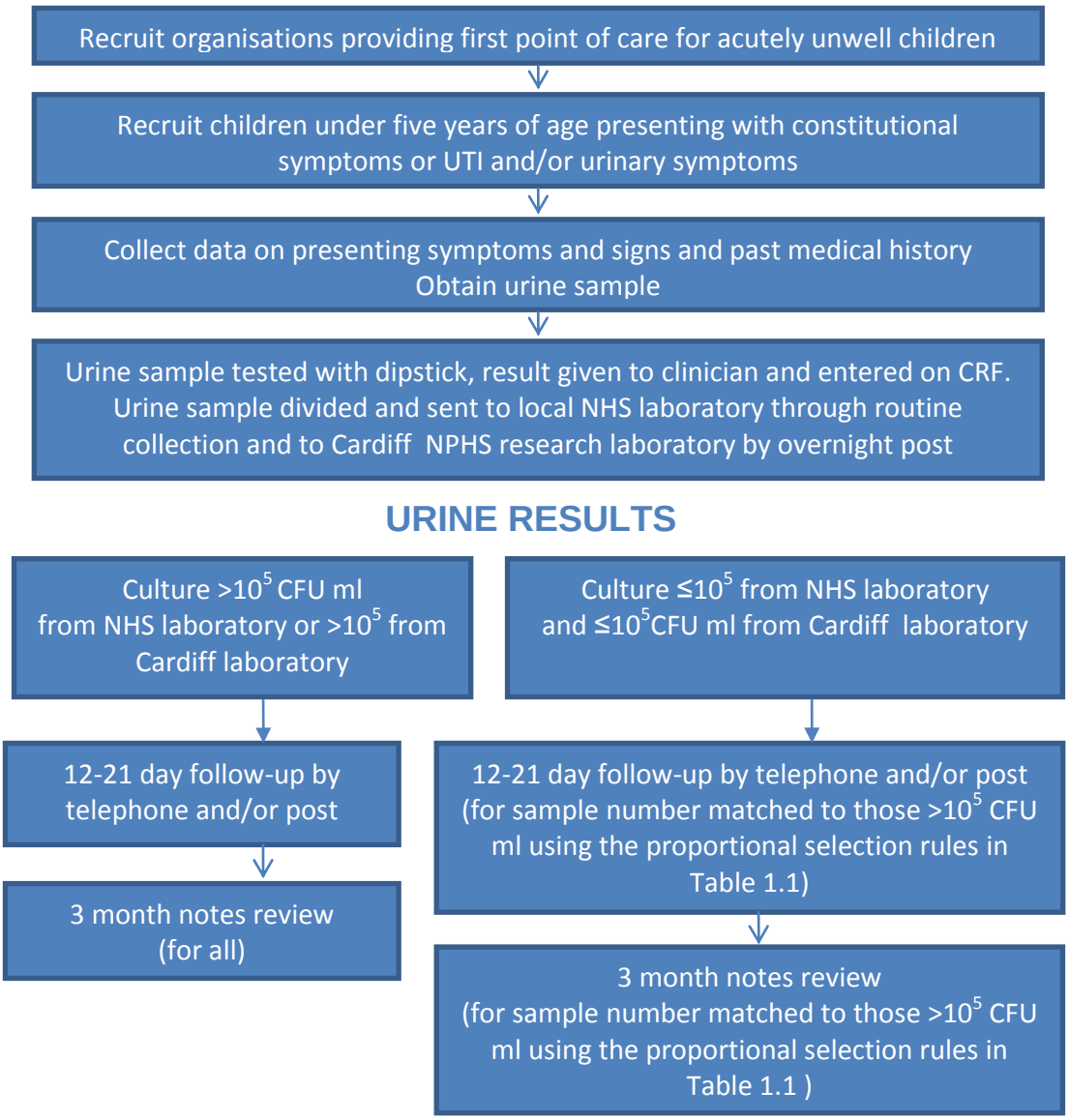
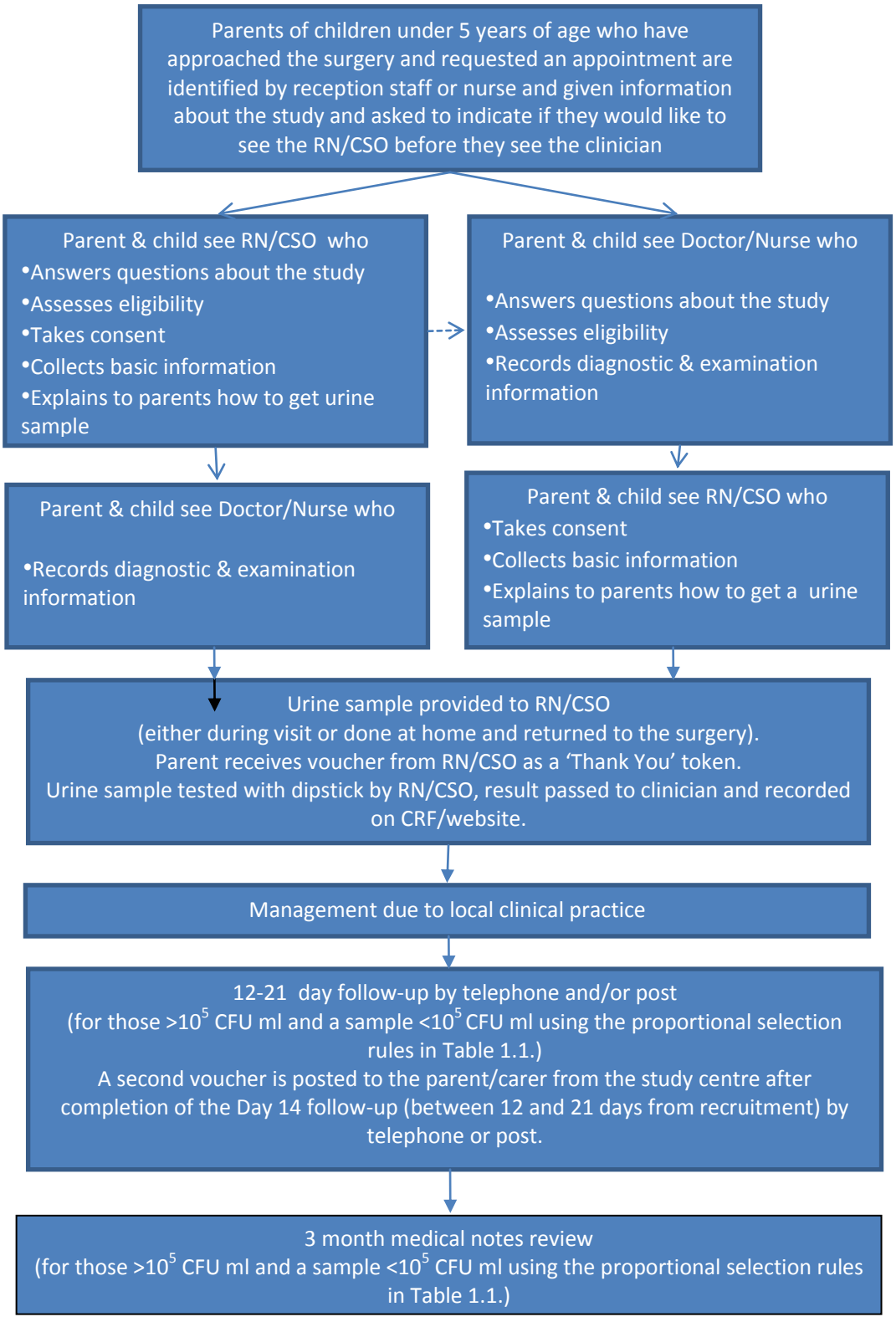


Table 1.1: proportional selection rules for DUTY follow-up:Category	**Definition**	**Location**	**Proportion to be sampled at Day 14**
> 10^5 CFU/mL	Pure or 1 predominant species	BOTH NHS lab and Cardiff Central lab	100% (All)
>10^3 and < 10^5 CFU/mL	Pure or 1 predominant species	Cardiff Central Lab	20% in total (*combination of both categories*)
>10^5 CFU/ML	2 or more species	BOTH NHS lab and Cardiff Central lab	
< 10^3 CFU/mL and 'No Growth'		BOTH NHS lab and Cardiff Central lab	10%

Participant Flow Diagram

Participant flow



1.3 Study Design Summary

DUTY is an observational study of the factors associated with UTI in children presenting in primary care. After completion of recruitment, patients will be split into development and validation sets for the development and testing of a new clinical prediction rule.

1.3.1 Study Aims

Primary Aim

To derive and validate a clinical algorithm for the diagnosis of UTI, in children aged before their fifth birthday presenting to primary care with an acute illness.

Secondary Aims

In children aged before their fifth birthday presenting to primary care, whose parents or carers have requested an appointment for an acute illness of less than or equal to 28 days:

1. To identify the additional value of a point-of-care dipstick urine test.
2. To model cost-effectiveness of one or more diagnostic algorithm guided strategies.
3. To compare contamination rates for two urine sampling methods.
4. To compare the results obtained from local NHS laboratories with the Cardiff Research laboratory
5. To explore the clinical significance of various threshold levels of positive urine culture.
6. To explore the clinical significance of mixed positive urine cultures.

1.3.2 Study Outcome Measures

Primary outcome

- To test the specificity and sensitivity of the algorithm using the reference standard of a pure/predominant growth of $>10^5$ colony forming units per millilitre (cfu/ml) of a single coliform or other recognised uropathogen as found in the NHS laboratory.

Secondary outcomes

- Effect of various diagnostic thresholds of positive culture for UTI on algorithm sensitivity and specificity.
- Sensitivity and specificity of point of care dipstick for identifying positive cultures.

- Symptom duration (measured between Day 12 and Day 21 from recruitment) and NHS contacts (measured at month 3).

1.3.3 Study Population

- Children aged before their fifth birthday, with an acute illness of equal to or less than 28 days presenting to primary care (GP practices, WICs, Out of Hours GP Cooperatives, CEDs or Polyclinics) in four UK regions (based around London, Bristol, Cardiff, Southampton).

1.3.4 Study Eligibility

- Children aged before their fifth birthday;
- Having approached the primary care site and requested an appointment for a new acute illness episode of equal to or less than 28 days.

1.3.5 Project Duration

- 3 years starting 1st January 2010.

2 Introduction

2.1 Background

Epidemiology and sequelae of UTI in children.

Acute illness in young children is one of the most common reasons for consulting health care worldwide. Reported rates of UTI in consulting children vary widely (from 1% to 9% depending on setting and inclusion criteria).[1,2,3,4,5] Most of this research has been hospital based: the prevalence of UTI in acutely ill children presenting to most of primary care is not known- no published study has yet systematically sampled urine from this population, although our smaller study, EURICA is ongoing. The diagnosis is difficult to make and may be missed in as many as 50% of children presenting to primary care.[6,7] UTIs in young children cause acute morbidity and recurrent symptoms that may indicate functional and anatomical abnormalities. In some young children, UTI may lead to renal scarring,[8] leading to poor renal growth, recurrent pyelonephritis, impaired glomerular function, early hypertension, end stage renal disease[9] and pre-eclampsia.[10,11,12] Some guidelines recommend aggressive, early antibiotic treatment for symptoms suggestive of UTI in young children to prevent renal scarring.[13]

Predictive value of symptoms and signs for UTI in young children.

A recent systematic review considered 8,837 mostly pre-verbal children from 12 studies.[6] Meta-analyses of data for the pre-verbal children showed that fever, non black race (likelihood ratio [LR] 1.4; 95% confidence interval [CI], 1.1-1.8), a history of a previous UTI (LR range, 2.3-2.9), temperature higher than 40°C (LR range, 3.2-3.3), and suprapubic tenderness (LR, 4.4; 95% CI 1.6-12.4) were the findings most useful for identifying those with a UTI. Uncircumcised boys were also more likely to have a UTI (summary LR, 2.8; 95% CI 1.9-4.3). Circumcision was the only finding with an LR of less than 0.5 (summary LR, 0.33; 95% CI, 0.18-0.63). Combinations of findings were more useful than individual findings (for temperature >39°C for >48 hours without another potential source for fever on examination, the LR was 4.0; 95% CI, 1.2-13.0; and for temperature <39°C with another source for fever, the LR was 0.37; 95% CI, 0.16-0.85). However, although individual symptoms and signs were helpful in the diagnosis of a UTI, they were not sufficiently accurate to definitely rule in UTI, although a combination of findings could identify infants with a low probability of UTI.[5]

This review is limited by: (1) included studies were set in the US private and emergency care system where consultation and investigation threshold differs from UK primary care which is free at the point of delivery; (2) children had to either already have symptoms of UTI or fever ≥38°C, so many subtle symptoms and signs may not have been considered; (3) urine sampling was by catheter or suprapubic aspiration (which is not feasible in UK primary care and from which any bacterial growth is regarded as significant); (4) diagnostic criteria used were different ($\geq 10^4$ cfu/ml) to UK practice; (5) the relationship between ethnicity and UTI could be confounded and; (6) none of the studies checked the external validity[14] of the findings, meaning that estimates of association could be inflated.[15]

Only one clinical algorithm derived from primary research for the diagnosis of UTI young children was identified. This was conducted in febrile girls <2 years in one US Emergency Department[16] and validated in a case-control study in a different, single Emergency Department.[17] They found that ≥3 of age <12 months; white race; temperature of ≥39°C; absence of any other likely source of fever; or fever for ≥2 days gave an area under the curve of 0.72, a sensitivity of 88% (95% CI 79% to 94%) with a false-positive rate of 70% (61% to 79%). Another study found that symptoms, signs and urinalysis were neither good at ruling in or ruling out UTI in children with neurogenic bladders.[18]

Additional value of dipstick testing in young children.

The recent HTA review found there was inadequate evidence on the diagnostic performance of dipstick tests for protein or blood.[19] The combination of a positive test for both nitrite and leucocyte esterase (LE) was most accurate for ruling in UTI (pooled LR+ 28.2 (95% CI: 17.3, 46.0)), and a negative test for both nitrite and LE was most accurate for ruling out UTI (pooled LR- 0.20 (0.16, 0.26)). The NICE UTI guideline development group concluded that there was insufficient evidence to recommend the use of dipstick urine tests for children <3 years.[20]

Diagnosing UTI in young children presenting in primary care.

The clinical diagnosis of UTI in young children is difficult because: (1) Pre-verbal (predominantly <3 years old) children cannot articulate symptoms and present with the same non-specific symptoms (e.g. fever, irritability, vomiting and poor feeding) when suffering from a wide range of illnesses;[21] (2) identifying dysuria and frequency in children wearing nappies (<3 years) is difficult; (2) obtaining urine samples is often frustrating and time consuming for parents/guardians and costly to the health service, [21,22] and; (3) NICE does not

recommend routine urine dipstick in children <3 years because of a lack of research evidence.[20] UTI diagnosis is therefore often delayed,[23] missed[7] or symptoms attributed to other causes (such as otitis media).[3]

Dramatic reductions in antibiotic prescribing for children with upper respiratory infections[24] may have reduced serendipitous treatment of undiagnosed UTI and the consequent prevention of renal scarring. NICE promotes early recognition and treatment to prevent short-term suffering and possibly serious long-term complications.[20] However; increased urine sampling will increase costs, consultation length and frequency of consultations in primary care. Rightly, clinicians will only increase their sampling rates if evidence shows this really does improve the identification of UTI among the many acutely unwell children consulting primary care.

Economic impact of UTI.

We know from studies of the General Practice Research Database (GPRD) that UTI is the 4th most common reason for prescribing antibiotics, accounting for approximately 8% of all antibacterial prescriptions.[25] Whilst the unit costs of laboratory testing and antibiotic prescribing are relatively low,[19] the *economic implications* of new clinical algorithms for urine sampling and testing may be substantial in young children because: (1) the large numbers of pre-verbal children who present with non-specific symptoms who might be candidates for urine sampling and testing; (2) the cost of subsequent diagnostic tests (e.g. ultrasound, Micturating Cystourethrogram (MCUG) and Dimercaptosuccinic Acid (DMSA) scans) used to further evaluate children with recurrent/atypical UTI;[20] (3) the substantial societal costs and utility detriments of a missed diagnosis that leads to rare but serious complications of UTI; and (4) the wider, long-term population impact of diagnostic algorithms on antibiotic prescribing and resistance.

The few economic evaluations of methods for diagnosing urinary tract infections in young children[19,26] have primarily evaluated 'which tests to use?' rather than 'who to test?'. The Health Technology Assessment report[19] evaluated 79 permutations of dipstick, cultures, ultrasound, and MCUG, and identified four testing strategies most likely to be cost-effective, although the optimal strategy differed by gender and age group. Current NICE guidance on testing strategies for UTI in children under age 3 is not based on evidence of cost-effectiveness.[20]

Summary

Good quality evidence regarding the externally validated predictive value of symptoms, signs and urinalysis for UTI in young children is urgently needed to help primary care clinicians better identify UTI. Furthermore, since obtaining urine samples is especially challenging in children <5 years, any resulting algorithm should be constructed to answer two separate questions. First, which children warrant urine sampling and second, can dipstick urinalysis help clinicians determine which samples should be sent for laboratory culture. The algorithm so developed should then be the subject of a validation study. In addition, since there is no evidence to help clinicians decide which urine sampling method to use, we propose an observational comparison of contamination rates for nappy pad and 'clean catch' sampling. Finally, since changes in the frequency with which urine samples are requested has implications for parents/guardians and the NHS, we propose to model the economic impact (from the NHS and societal perspectives) of current vs. diagnostic algorithm guided diagnosis and management with respect to the cost per correctly identified UTI, cost per symptomatic day avoided and the cost per quality adjusted life year.

2.2 Rationale

- NICE guidelines[20] state that good quality evidence regarding the externally validated predictive value of symptoms, signs and urinalysis for UTI in young children is urgently needed to help primary care clinicians better identify UTI.
- DUTY will provide novel, clinically important information on the epidemiology and presentation of childhood UTI that will be used to develop a feasible and cost effective clinical tool to help primary care clinicians in the UK and worldwide improve the efficiency of diagnosing UTI in young children. This will help minimise acute suffering and may help prevent serious long-term complications. A web based decision aid (similar to those currently used to estimate cardiovascular risk) could be made available for routine use in the NHS via the National Library for Health. DUTY will improve understanding of the optimum bacterial concentration for UTI in young children and provide comparison data on nappy pad and 'clean catch' sampling methods.

2.3 Research Question

What is the best way to diagnose a urinary tract infection in young children aged less than 5 years of age presenting to primary care with an acute illness of equal to or less than 28 days duration?

3 Study Objectives

3.1 Research Aims

The overall aim of the DUTY study is to derive and validate an algorithm for the diagnosis of UTI in children less than 5 years of age presenting to primary care with an acute illness. The algorithm will be constructed in two stages: identification of children at risk (in whom a urine sample should be obtained) and determining the added value of point-of- care urine dipstick testing. The findings may be combined to produce an overall algorithm.

3.2 Research Objectives

1) To develop an algorithm that accurately identifies children presenting in primary care with an acute illness in whom a urine sample should be obtained, based on socio-demographic factors, medical history, symptoms and signs.

2) To assess whether dipstick urinalysis for nitrite, leukocyte esterase, protein, blood and glucose gives additional diagnostic information to objective (1) in the identification of urine samples that should be sent to the laboratory.

3) To model cost-effectiveness (cost per correct diagnosis of UTI, cost per symptomatic day avoided, and lifetime cost per quality adjusted life year, including potential long term complications of UTI) from NHS and societal perspectives of one or more diagnostic algorithm guided strategies.

4) To compare contamination rates for nappy pad versus "clean-catch" urine sampling methods.

5) To explore the impact of two different urine categories on symptom duration (measured between Day 12 and Day 21 from recruitment) and the number of NHS contacts (at 3 months): (i) urines categorised as 'contaminated' by the NHS laboratory (compared with NHS laboratory urines categorised as 'no growth' and: (ii) bacteriuria concentrations $>10^4$ cfu/ml but $<10^5$ cfu/ml with bacteriuria concentrations $>10^3$ but $<10^4$ cfu/ml, both defined by the Cardiff NPHS research laboratory.

4 Study Design

4.1 Study Outline

DUTY is a multicenter, diagnostic accuracy study (research objectives 1 and 2 above), decision analytical model (3), nested analytical (4) and follow up study (3 and 5) to derive and validate a cost effective algorithm for the diagnosis of UTI in children aged less than 5 years presenting to primary care with an acute illness.

Children will be recruited from primary care, being any NHS health service providing first-point-of-contact face-to-face advice for parents/guardians of unwell

children (GP practices, WICs, Out of Hours GP Cooperatives, CEDs and Polyclinics). Children will be eligible if they are aged before their fifth birthday and are presenting with a new acute illness episode of less than or equal to 28 days in duration, and if the parent or carer has approached the primary care site and requested an appointment for the child's acute illness. A CRF will be completed for all eligible, consented children and a urine sample obtained. The prevalence of UTI will be determined on laboratory culture.

An algorithm will be derived and validated.

4.2 Study Period

The study will last for three years from 1st January 2010.

4.3 Frequency and Duration of Participant Follow-Up

- ***Recruitment***

At the initial consultation (and time of recruitment into study), each participant will be seen by a RN/CSO from the local recruitment centre who will obtain consent, complete the CRF and obtain a urine sample. Children will be eligible for the study if they meet the eligibility criteria at the point of recruitment and their parents or carers have approached the primary care site and requested an appointment for the child's acute illness. Participants will then be seen by the clinician who will record examination findings, working diagnosis and treatment on the CRF. Clinical management of the participant will be according to the clinician's normal practice. For most participants this will complete their active involvement in the study.

- ***Telephone and/or postal follow up between Day 12 and Day 21 from recruitment***

Each Research Site (Bristol, Cardiff, London and Southampton) will telephone the parents/carers of all children with culture positive (as defined by the NHS laboratory at $>10^5$ cfu/ml or by the Cardiff research laboratory at $>10^5$cfu/ml, estimated to be a maximum of 8% children per research site respectively) and an equivalent number of children with culture negative urines, who will be selected according to the proportional rules in Table 1.1. This contact will consist of a telephone interview (or, if the parent cannot be contacted by telephone, a postal version of this questionnaire will be sent out enclosing a prepaid return envelope) to record symptom duration (particularly asking if the child responded to treatment <48 hours since NICE has identified this as a marker of 'atypical UTI' and recommends DMSA and MCUG in such children) and resource use (e.g. repeat

primary care visits, NHS Direct contacts, secondary care costs and personal time and travel costs) the 14 days following recruitment.

On completion of the Day 14 follow-up by telephone interview and/or postal questionnaire, a second £5 High Street voucher will be sent to the parent/carer to thank them for their time.

- ***Month 3 notes review***

Each Research Site will conduct a primary care notes review, for all those selected for telephone and/or postal follow up as above, for the number of primary care contacts (except NHS Direct). A secondary care notes review will be conducted for all children with a UTI (as determined by the NHS laboratory), and comparator 'no bacterial growth' children (likely to be no more than 10 per centre) for secondary care costs (out-patient attendances, admissions and investigations (e.g. ultrasound, MCUG or DMSA scans).

4.4 Study Outcomes

4.4.1 Primary Outcome

- To test the accuracy of the algorithm using the reference standard of a pure/predominant growth of $>10^5$ colony forming units per millilitre (cfu/ml) of a single coliform or other recognised uropathogen.

  (For the primary analysis, samples containing $\leq 10^5$ cfu/ml or a mixed growth of ≥2 bacterial species (contaminants) will be regarded as negative samples).

4.4.2 Secondary Outcomes

- For research objectives 1, 2 and 3 (section 3.2), we will explore the effects on algorithm sensitivity, specificity and costs of using $>10^5$, $>10^4$ and $>10^3$ cfu/ml of a pure/predominant growth of a single coliform or other recognised uropathogen. Differences with local laboratory results will also be explored.
- For objective 3 (section 3.2), we will measure symptom duration and resource use (e.g. repeat primary care visits, NHS Direct contacts, secondary care costs and personal time and travel costs) by telephone and/or postal follow-up between Day 12 and Day 21 from recruitment. Resource use will also be measured at 3 months to assess interim NHS and societal costs.
- For objective 4 (section 3.2), contamination will be defined as a mixed growth of >2 bacterial species at $>10^5$ cfu/ml.

- For objective 5 (section 3.2), we will measure the number of NHS contacts from the primary and secondary care medical records, and where available, the results of repeat urine samples.

5 Participant Selection

5.1 Inclusion Criteria

Children will be included if:

- Aged before their fifth birthday.
- Presenting at an NHS primary care site (GP practices, WICs, Out of Hours GP Cooperatives, CEDs and Polyclinics).
- Presenting with an acute (≤28 days) illness as the main reason for the parent / carer to have approached the primary care site and requested an appointment.
- Presenting with at least one 'constitutional' symptom or sign identified by NICE[20] as a potential marker for UTI – that is, fever, vomiting, lethargy/malaise, irritability, poor feeding and failure to thrive *and/or* at least one urinary symptom identified by NICE[20] as a potential marker of UTI – that is, abdominal pain, jaundice (children <3 months only), haematuria, offensive urine, cloudy urine, loin tenderness, frequency, apparent pain on passing urine and changes to continence.

5.2 Exclusion Criteria

Children will be excluded if:

- Aged 5 years and above; illness longer than 28 days duration; no urinary or constitutional symptoms as defined by NICE[20] and listed above; known neurogenic (e.g. spina bifida) or surgically reconstructed bladder or urinary permanent or intermittent catheterisation (for whom different bacterial concentration cut points are used).
- Parents/guardians are unable or unwilling to assist with study.
- Presenting with trauma as a predominant present concern.
- Taking any antibiotics in the last 7 days.
- Taking immunosuppressant medication (e.g. anti-rejection drugs, oral or intramuscular steroids or chemotherapy).
- Already recruited into the DUTY study.
- Involved in current research or have recently (within 28 days) been involved in any research prior to recruitment.
- There will be no recruitment to the study after the last transport of the day has departed from that primary care site on Fridays.
- For recruitment at A&E settings only: children will not be eligible if their presentation at A&E is as a direct result of GP referral.

The aim of this study is to have as broad inclusion criteria as possible. Therefore children requiring hospital admission with other 'obvious' causes for their symptoms such as otitis media or bronchiolitis, with a history of UTIs and known abnormalities of the urinary tract, learning difficulties, or re-consulting for an existing illness are all included as long as none of the exclusion criteria apply.

We will include parents/guardians who speak other languages in our study. Language requirements will be assessed on an individual primary care site basis. In our site set-up discussions we will ask each individual site about dominant languages in their patient population. We will then provide Parent Information Sheets and Consent forms translated into the languages as required by the individual practices, and for rarer languages translational services will be provided.

We will comply with Welsh language requirements and the Research Participant Information Sheet, Consent form and any other required participant documentation will be available in Welsh.

6 Recruitment

6.1 Recruitment Process

All primary care sites will display posters detailing the DUTY study.

6.1.1 GP Practice Recruitment

Between 80 and 100 practices will be recruited by each Research Site. Each research site needs to recruit, on average, 1625 - 1750 patients in order to give a whole study recruitment population of 6,500 to 7,000. Experience from a previous study (EURICA) shows that most primary care sites can recruit between 50 and 100 children aged less than 5 years in a year.

Experience from the best recruiting sites in EURICA supports the following approach strategies for DUTY. Where possible, primary care sites will mention the study to parents/guardians of children who are less than 5 years old when they phone for an appointment and ask them to come to the surgery 15 minutes early for further information about the study. Where the study was not raised at the time of making the appointment, parents/guardians of children in this age group already booked in may be phoned and told about the study and invited to attend a little earlier. If they cannot be contacted by telephone, they will be approached on arrival at the surgery, given information sheets about the study, and asked if they would be happy to see the RN/ CSO first to discuss the study.

The RN/CSO will discuss study participation and check eligibility criteria. Parents/guardians agreeing to participate will provide written, informed consent. Consenting parents/guardians will be asked to obtain a urine sample. The CSO will give the appropriate equipment and explain how to collect this. Assistance will be given where necessary. If the parent/guardian wishes to see the GP before consenting, then this can happen, with them returning to the CSO afterwards to complete consent and study materials.

Where possible the Research Nurse/Clinical Studies Officer (RN/CSO) will recruit the participant whilst they are waiting to see the doctor, in order that the participant is not delayed. However, if the participant is getting delayed and it would be more convenient for them, or a clinician has discussed the study with a potential applicant when the RN/CSO is not present in the waiting room (e.g. with another parent/child), the RN/CSO will offer to visit the participant later the same day at their home. The parent/guardian will be asked to sign a form consenting for their contact details to be passed to the RN/CSO face to face, by telephone or fax so that the RN/CSO can subsequently contact the parent/guardian. These contact details will be destroyed as soon as the parent/guardian has been contacted.

6.1.2 Collection of Urine Samples

Suprapubic aspiration (SPA) carries the least risk of contamination[27] but is invasive and not appropriate or feasible for widespread use in UK primary care. The risk of contamination is low with clean-voided midstream urine (CVU) or 'clean catch' samples compared to SPA samples.[19] However, obtaining clean catch samples can be difficult in children in nappies, and parents/guardians find nappy pads or urine bags preferable.[28] These may be suitable alternatives to SPA,[19] but there are few data comparing CVU with pad samples. Thus, the preferred primary care method is the CVU and NICE recommended pad or bag when CVU is not possible.[20] DUTY will follow this NICE recommendation for collecting urines. Urine sampling can be underway whilst the CSO completes the study 'Case Report Form' either on paper or electronically via the study website.

The child can then see the responsible treating clinician who will treat the child according to their usual best practice. The clinician will enter relevant examination findings on the Case Record Form (CRF) or directly on a DUTY secure website. The child will then return to the CSO who will retrieve the urine sample, establishing the method (CCU vs. Nappy Pad) by which it was obtained. The

RN/CSO will test this with a urine dipstick. Urine sampling method and dipstick results will be recorded in the CRF/website.

Every effort will be made to obtain the urine sample while the child is at the recruiting site. However, if this is not possible, the parent/guardian will be given the necessary equipment and advice on taking the sample at home. They will be advised to store it in the fridge and return it to the surgery as soon as possible. The RN/CSO will telephone parents/guardians the next day to remind them to return the sample. Sites and the research team will be able to check if samples have been received at practices (or local laboratories) by logging onto the secure website, which will monitor (in real time) the location of specimens. Once a sample is received in the practice, the practice staff or CSO will test it with a dipstick, pass the result to the responsible clinician, record the results and sampling method on the paper CRF/website. The sample will then be split, labelled and sent to both local and central laboratories as detailed above. The study will not interfere with the usual processing of urine results or the clinician's routine management.

6.1.3 Walk in Centre/ Polyclinic Recruitment

Walk in Centres (WICs) located in urban areas see large numbers of young children with acute, undifferentiated illnesses. For example, the South Bristol WIC estimates 2,500 contacts for acute, non-traumatic, illnesses in children <5 years annually. Recruitment at WICs will operate in the same way as GP practice recruitment up to and including obtaining the urine, and adding the sampling method and dipstick results to the paper CRF/website. The urine sample will either be collected direct from the WIC or delivered to the child's GP practice by the parent/guardian, or RN/CSO depending on local specimen collection arrangements, where it will be processed as for GP practice derived samples (see below). All clinical results will be sent to the child's GP in the usual way. Although the location and integration with existing services of Polyclinics has yet to be finalised in many areas, we anticipate DUTY recruitment methods will follow either the GP practice or WIC model.

6.1.4 Children's Emergency Department Recruitment

Large numbers of acutely unwell pre-school children attend Children's Emergency Departments (CEDs) as a first point of contact with fever and other symptoms which merit inclusion in the DUTY study, particularly outside normal working hours. Over 5% of all attendances at a UK CED are due to fever,[29] which represents approximately 1,400 children annually in each of Bristol, Cardiff and Southampton, and more in London. The majority of these are <5 years old. All

children attending the CED are rapidly triaged by experienced NHS staff, who will provide a DUTY information sheet and additional verbal information to the parents/guardians of all eligible children and inform parents/guardians about the need for urine collection. The study will then be discussed with the parent(s) /guardian(s) by the treating CED doctor, who will obtain written consent from all parents/guardians willing to participate. The collection, dipstick and laboratory analysis of urine samples in pre-school children with fever or relevant symptoms (usually using a clean catch method) is standard practice in most CEDs, so minimal change to current practice is required to accommodate the DUTY study.

As a consequence of their underlying illness (e.g. investigation of fever, or for the treatment of pneumonia), a higher proportion of DUTY children recruited at CED sites will be admitted to hospital than children recruited at other primary care sites (e.g. GP practices). It is anticipated that most admissions under these circumstances will be recorded as an expected and unrelated SAE. It is likely that the DUTY study will have a relatively high number of expected and unrelated SAEs as a consequence of recruitment in CED settings.

6.2 Registration & Informed Consent

Reception Staff will give a study information pack to all parents/guardians of consecutive children aged before their fifth birthday who have approached the site and requested an appointment for care, and invite them to talk to a RN or CSO about the study. The parent/guardians will be given time to read the study information in the waiting room. Parents/guardians not interested in hearing more about the study at this stage will have no further contact about the study.

The RN or CSO will answer any questions about the study. Further time will be given to the parent/guardian for reading the material if required, and a further opportunity for asking questions. If the child is eligible for the study, consent will be obtained from those parents/guardians willing for their child to take part. Consent will be taken by a RN or CSO trained in taking consent and in study procedures.

The CRF will be completed for all consented patients. This will be a short medical history including recent antibiotic use and other potential risk factors for UTIs and resistance, and examination findings. An outline of the domains which will be covered in the CRF can be found below in the next section. The CSO will record history details and some examination findings (including temperature, pulse, respiratory rate oxygen saturation and capillary refill time) and obtain a urine sample.

The sample will be tested with a dipstick by the RN or CSO and results given to the responsible clinician, and recorded in the CRF (tests include presence of blood, protein, leucocyte esterase, nitrate).

The sample will then be labelled and processed as described in the study procedures section.

After the child has been seen by the RN or CSO (and if they have not already done so), they will then see the responsible treating clinician, who will manage them according to their normal practice, and complete the examination and management details of the CRF.

6.3 Non-registration

A screening log of all children under the age of five attending for care will be compiled. Details will be recorded of whether they were approached about the study or not, eligibility and whether consent was given or declined. No identifiers will be recorded on this log.

6.4 Withdrawal & Loss to Follow-Up

In the majority of cases the only active participation of participants is at the initial consultation, and withdrawal from the study in most cases is unlikely.

Several attempts will be made to contact parents/guardians by telephone, however if unsuccessful the postal questionnaire will be posted to participants with a stamped addressed envelope for return. If a telephone interview has not been achieved and the postal questionnaire has been sent but is not returned within two weeks of sending the patient will be considered as lost to follow-up.

Parents/carers of children selected for follow-up who have completed the telephone interview and/or postal questionnaire will be sent a second £5 High Street voucher as a thank you for their time.

6.5 Payment

Following collection of the urine sample, the parent/guardian will receive a £5 High Street Voucher from the RN/CSO as a 'Thank You' token for taking part. Parents/guardians of children selected for follow-up will be sent a further £5 High Street Voucher by the Study Administrators on completion of the telephone interview and/or postal questionnaire.

7 Study Procedures

7.1 Data Collection and Case Record Form (CRF)

The web based CRF will contain as many of the known and potential features associated with UTI as are possible without overly compromising the speed and simplicity of completion. **Four sections** will facilitate data entry by different personnel (Research Nurse/CSO/Responsible Clinician) so as to minimise the burden to healthcare professionals undertaking emergency clinics (e.g. 'same day primary care surgeries'):

1. *RN/CSO*: Socio-demographic data (e.g. date of consultation, name, address to include postcode (to assign an index of socio-economic deprivation), contact telephone number/s, ethnicity,[16] date of birth,[20] and gender,[20]).

2. *RN/CSO*: Known previous medical history (e.g. previous UTI, circumcision,[6,31] child or family history of vesico-ureteric reflux,[32] other abnormalities of the urinary tract, learning difficulties, details of prior surgery, other co-morbidities, recent and previous long term use of medicines, including antibiotics.

3. *Responsible clinician*: Working diagnosis; the severity and where appropriate (e.g. fever[16]) duration of symptoms and signs. In addition to the 'constitutional' and 'urinary' study eligibility symptoms defined by NICE,[20] we will collect information regarding the clinician's global assessment of illness severity,[33] respiratory and gastro-intestinal symptoms and signs, and the symptoms and signs proposed to NICE to distinguish 'typical' from 'atypical' UTI (e.g. poor urinary flow and abdominal mass). Primary care clinicians will be asked to record the child's management, including antibiotic use and immediate referral to secondary care since we will make every effort to obtain urine results from these children. Finally, for the economic analysis, we will ask clinicians to state what their management would be if the patient were not enrolled in the DUTY trial (no urine test or treatment for UTI / urine test / treat for UTI). This will provide information on the 'clinician judgement' diagnostic strategy that will be a comparator for other diagnostic algorithms.

4. *Practice nurse/Triage Nurse/RN/CSO*: Urine sampling method (including if SPA or catheter in CED) and urinalysis results with date, time of testing, with a prompt to inform the responsible clinician of dipstick result and confirmation that the sample has been sent to the laboratories.

7.2 Collecting Urine Samples and Dipstick Testing

Consenting parents/guardians will be asked to obtain a urine sample. The RN or CSO will give the appropriate equipment and explain how to collect this. Assistance will be given where necessary. Urine samples will be obtained using the 'clean catch' (preferably) or 'nappy pad' method as recommended by the recent NICE guidelines[20]. Urine sampling can be underway whilst the CSO/RN completes the study CRF/website.

The RN or CSO will retrieve the urine sample, establishing whether obtained by clean catch or nappy pad. The RN/CSO will test the urine sample with a urine dipstick (Urine Dipstix 8SG, Williams Medical) provided by the study. Urine sampling method and dipstick result will be recorded on the CRF. The urine sample will then be split in half if sufficient quantity is available with the priority fraction being sent to the NHS laboratory and the second 'research' fraction being sent to the Cardiff NPHS SACU laboratory.

If a sample is not obtained during this visit the parent/guardian will be asked if they could take this at home, refrigerate the sample and return it to the primary care site or GP if recruited at a primary care site without routine laboratory transport.

7.2.1 Maximising Urine Samples

Obtaining the urine samples will be challenging, and a suboptimal return rate will diminish power and increase risk of bias. Therefore: (1) we will monitor the location of urine specimens using a web-based system. Contact details and clinical data for recruited children will be logged onto the secure study website.

Dipstick urinalysis data may be added after the clinical data and will provide a record of the urine having been obtained. This will allow both the research team and participating sites to identify and call parents/guardians of children who have not yet submitted urine samples.

Both the Research and NHS laboratories will also record the arrival of, and results from, the specimens on the website and; (2) each centre will employ a dedicated study RN and a CSO to assist practices with obtaining urines.

7.3 Laboratory Processing of Urine Samples

As not all the laboratory diagnostic services supporting participating DUTY sites report bacteria $\leq 10^5$ cfu/ml, we plan to split all urine samples at the recruiting sites, with the first (priority, minimum 1ml) sample sent in the usual manner to the usual NHS laboratory for routine diagnostic processing, and the second sample sent by post to the NPHS Research Laboratory in Cardiff.

The first part of the urine will be put into containers recommended by the local NHS laboratory and labelled with the child's details on special DUTY labels provided in the patient packs. Similar DUTY labels will be adhered to the DUTY study specific microbiology form and the sample sent to the local NHS laboratory

using the site's normal method of transport. Any samples not collected within 4 hours will be refrigerated and processed in less than 36 hours.

The other urine part (whatever is left after 1 ml has been reserved for the local laboratory in the case of small samples) will be put into a special DUTY container. This will be labelled with the child's unique DUTY study ID number and date of birth but no identifiable information, and sent by 1st Class Royal Mail using Post Office approved Safeboxes™ to the Cardiff Laboratory, a method that meets legal requirements and has been successfully used before. Clinicians will receive reports from their local laboratory in the usual way. They and the local NHS laboratory manager will also be informed of any positive culture results from the Cardiff NPHS research laboratory for those patients for whom the local laboratory sample is discrepant i.e. culture negative, contaminated or not processed, and this discrepancy is considered to be clinically significant by the research laboratory microbiologists.

7.3.1 Processing of Urine Samples by NHS Laboratories

NHS laboratories will be informed of the study and educational materials provided before patient recruitment. They will be reminded of the tasks requested by the DUTY study by the use of web-based laboratory data collection forms. These tasks are:

1. Log the date and time of specimen arrival on the DUTY secure website.

2. Process the urine and report the result back to the requestor using their own Standard Operating Procedures (SOPs) and Laboratory Information Management Systems (LIMS).

3. Provide a copy of the routine (paper and/or electronic) report to the research team and additional information requested on the DUTY request form. Since laboratories vary in their SOPs, not all of the following will be available (hence the need for Cardiff research laboratory processing) but: microscopy for white and red cells; quantification and purity of bacterial growth; and speciation will be requested. Laboratories will be asked to transcribe this information onto the DUTY web site in order to activate laboratory payment.

4. Keep any isolates from urines with $>10^5$ CFU/ml in pure/predominant growth for referral onto the Cardiff research laboratory at the end of the study. These should be stored at temperatures of -70^0C.

7.3.2 Processing of Urine Samples by the Research Laboratory (Cardiff)

The 'Cardiff Research Laboratory' refers to the Public Health Wales Microbiology Laboratory based in Cardiff which had a similar role in the previous EURICA study and has experience in supporting other primary care UTI studies.

1. Urines will be sent overnight by Royal Mail post by the participating sites. Boric acid may be used to stabilise bacterial counts if initial data on bacterial count stability shows this to be needed.
2. On receipt at the Cardiff laboratory, the urine sample will be tested with a dipstick and also tested for low count glucose. Then spiral plating on blood agar and CLED agar will be used to quantify bacteria $>2x10^3$ CFU/ml and $<10^{10}$ CFU/ml.
3. The bacteria will be identified to species level and stored at -70°C. The urine will be stored frozen.
4. Results will be recorded on a designated laboratory worksheet and entered into the on-line database
5. The study team will also receive laboratory results from the local NHS laboratories.

7.4 Laboratory Definition of UTI

DUTY will collaborate with a large number of laboratories that use the standard of $>10^5$ cfu/ml[35] and study results will need be applicable to current UK practice. A lower threshold, for example, of $>10^4$ cfu/ml used in US studies, may generate false positives and inappropriate 'medicalisation' of illness. Nevertheless, DUTY presents an opportunity to explore the effects of different bacterial concentration thresholds and species on algorithm sensitivity and specificity, and to compare various laboratory thresholds with clinical diagnoses and outcome. This will help both to better meet our main study objective as well as fill an important gap in the evidence base to support common clinical and laboratory diagnostic practice, since current practice is based on old studies of UTI in pregnancy[34 35] and some bacterial species (e.g. coagulase negative staphylococci) achieving diagnostic growth concentrations are unlikely to be urinary pathogens. UTI in DUTY will therefore be defined as $>10^5$ cfu/ml of a coliform or other recognised uropathogen.

7.4.1 Minimising effects of sample contamination and assessment of asymptomatic bacteriuria

Contamination of urine (a cultured organism from a source other than the urinary tract) can lead to false positives: a rate at 7.2% was identified by comparing pairs of urines obtained by different methods from 203 children.[36] All nine (5.4%) children in this study with a mixed culture $\geq 10^5$ cfu/ml of uropathogens (a heavy mixed growth) in their first sample had a UTI excluded in the second.[36]

In addition, bacteria at $\geq 10^6$ cfu/ml have also been found in the urine of approximately 1.5% of young, asymptomatic, children when screened using the 'gold standard method', supra-pubic aspiration,[37] and most did not experience long term sequelae.[38] So, distinguishing UTI from asymptomatic bacteriuria and bacterial contamination is difficult, and could lead to spurious associations between symptoms (e.g. diarrhoea) and apparent 'UTI' that is in reality contamination or potential harmless asymptomatic carriage. Clinicians use the presence of UTI symptoms to help interpret culture positive results but this leads to incorporation bias. In DUTY, we could restrict recruitment to those children with currently recognised symptoms of UTI, but since the purpose of DUTY is to determine the strength of association between currently recognised as well as *currently unrecognised* symptoms/signs and UTI, it is important that eligibility criteria are as 'open' as possible (and that a prospective cohort, as opposed to retrospective case-control, design is used), but without including children in whom a positive culture is unlikely to be clinically relevant (e.g. a well child with conjunctivitis). Therefore, DUTY will recruit children with constitutional and/or urinary symptoms and make the assumption that the presence of pathological bacteria $>10^5$ cfu/ml growth on culture of their urine is clinically significant. This could result in more urine samples being tested and more children receiving antibiotics than is strictly necessary, but carries the benefit that more UTIs would be identified and treated promptly.

To minimise contamination, we will use clean catch where possible. Where not possible, the child's perineum will be thoroughly cleaned and we will use pads for <30 minutes, as described by Liaw *et al*.[28] When found, the impact of three factors on symptom duration (measured between Day 12 and Day 21 from recruitment) and the number of NHS contacts at 3 months: (i) clinician diagnosis (UTI vs. plausible alternative diagnosis); (ii) UTI vs. contamination (as per standard NHS laboratory reporting); and (iii) differing bacteriuria concentrations (measured in the Cardiff research laboratory).

7.5 Patient Follow-Up

7.5.1 Telephone and/or postal follow up at 14 days, contact between Day 12 and Day 21 from recruitment

Each RS will contact the parents/carers of all children with NHS or Cardiff research laboratory culture positive UTI ($>10^5$ cfu/ml) and an equivalent number of children with NHS and Cardiff laboratory non-positive urine culture results (selected at random using the proportional selection rules in Table 1.1). This contact will in the first instance consist of telephone interview (or, if unable to make telephone contact with the parent/carer, a postal questionnaire will be sent out with a prepaid return envelope) to record symptom duration (particularly asking if the

child responded to treatment <48 hours since NICE has identified this is a marker of 'atypical UTI' and recommends DMSA and MCUG in such children) and resource use (e.g. repeat primary care visits, NHS Direct contacts, secondary care costs and personal time and travel costs).

7.5.2 Month 3 notes review

Each Regional Centre will conduct a primary care notes review for all children with NHS laboratory or Cardiff research culture positive UTI (maximum 150) and an equivalent number of randomly selected children (as followed up between Day 12 and Day 21 from recruitment, i.e. selected at random using the proportional selection rules in Table 1.1) NHS and Cardiff laboratory non-positive urine culture results for the number of primary care contacts (except NHS Direct). A secondary care notes review will be conducted for children with a culture positive result and a sample of culture negative children (likely to be no more than 10 per centre) for secondary care costs (out-patient attendances, admissions and investigations (e.g. ultrasound, MCUG or DMSA scans).

8 Adverse Events

8.1 Definitions

Adverse Event (AE):

Any untoward medical occurrence in a study participant.

Serious Adverse Event (SAE):

Any untoward and unexpected medical occurrence or effect that: Results in death; is life-threatening (refers to an event during which the participant was at risk of death at the time of the event; it does not refer to an event which might have caused death had it been more severe in nature), requires hospitalisation, or prolongation of existing hospitalisation; results in persistent/significant disability or incapacity; is a congenital abnormality or birth defect.

We do not foresee any related adverse or related serious adverse events happening as a result of this research, as the study involves only a non-invasive urine test which is often part of the routine clinical care of patients. We do, however, foresee a relatively high number of unrelated and expected SAEs as a consequence of hospital admissions of acutely unwell children, especially (though not exclusively) for children presenting to recruiting CED sites.

8.2 Evaluating and Reporting Adverse Events

We will ask parents/guardians and primary care sites to inform the Study Manager of any SAE. The DUTY Study Manager and/or the Chief Investigators will assess the nature of the SAE, for seriousness, causality and expectedness. Following the initial report, follow up data may be requested by the DUTY Study Manager. All reports will be submitted to the SSC. Where the SAE is both related and unexpected the DUTY Study Manager will notify the main REC within 15 days of receiving notification of the SAE.

9 Study Analysis

9.1 Sample Size Calculation

We are particularly well placed to estimate the required sample size, given our experience with the EURICA study, which has already recruited approximately 680 children and found a UTI prevalence rate in children aged under 5 years of 5%. We have considered first the strength of association between candidate predictors (symptoms, signs or dipstick results) and UTI as well as the precision of the final algorithm's sensitivity for the detection of UTI. Taking the most conservative assumptions, i.e. candidate predictors present in 10% of children and an overall UTI prevalence of 2%, 3,000 urine sample results are required to detect an odds ratio of 2.4 with 80% power and a two-sided alpha of 5%. With an overall prevalence of UTI of 2%, an algorithm sensitivity of 80% and 3,100 urines, the 95% confidence interval (CI) will be no more than +/-10%. We propose to recruit 4,000 children with a target of recovering urines from at least 77.5% for algorithm derivation and a further 2,000 children for validation.

9.2 Amendment to Sample Size Calculation made February 2012 (after 22 months of recruitment)

The initial sample size calculation for this study was based on the following considerations:

1. Consideration of the strength of association between candidate predictors (symptoms, signs or dipstick results) and UTI as well as the precision of the final algorithm's sensitivity for the detection of UTI. Based on conservative assumptions (candidate predictor items present in 10% of children and an overall UTI prevalence of 2%, we estimated that 3,000 urine sample results would be required to detect an odds ratio of 2.4 with 80% power and a two-sided alpha of 5%. With an overall prevalence of UTI of 2%, an algorithm sensitivity of 80%, the 95% confidence interval (CI) would be no more than +/-10% if we had 3,100 urine samples with linked clinical data for final analysis.

2. Likely urine retrieval rates. Not all recruited children are able to provide urine samples. For the purposes of deriving the clinical prediction rule, we aimed to recruit 4,000 children, with a target of obtaining urines from at least 77.5%.

3. We estimated that a further 2,000 children would need to be recruited to obtain sufficient additional urine samples with linked clinical data to validate the clinical

prediction rule in an independent sample. Established practice is that the development sample should be larger than the validation sample.

4. Consideration of the maximum achievable sample size given likely limits on budgets and eligible patient populations. At the start of the study, the investigators felt that aiming for a larger sample size, even though this would have been desirable from the point of view of statistical precision, might not have been feasible or affordable. Overall therefore, the target for the number of urine samples from clinically well-characterised recruited children was 4,650.

At the time of writing (**March 2012), patient recruitment (May 2010-April 2012) is proceeding ahead of target. The rate of patient recruitment has meant that the DUTY study achieved a recruitment population of 6,900 patients, and a primary outcome sample of 4,650 urines with NHS culture results entered online, on 29 March 2012 2012.**

The preference of the DUTY investigators and of the host institution is to continue recruitment until the end of the scheduled period (i.e. to 30 April 2012), in order to maximise the statistical power of the sample. More patients will mean we are able to provide clinicians and patients with more precise estimates to guide care. Based on the above assumptions, if we continue recruiting until the end of the originally planned recruitment period, this could lead to a final recruitment sample of an estimated 7500 patients, with approximately 6000 – 6500 NHS urine culture results entered online, i.e. an additional 15 % compared to the original sample size. We would ask that the upper limit of recruitment be removed to allow for as many participants as possible, thereby maximising study power and precision. Urine positivity appears to be close to our originally assumed 2%, though this does depend on the precise definition of UTI adopted.. Nonetheless, these figures imply that the development of the clinical prediction rule will be based on fewer than 200 positive urine samples. Given the complexity of the statistical analyses, large number of possible predictors and the need to account for some missing data in predictor variables (which was not accounted for in the original sample size calculations), increasing study power will not be futile or merely academic.

In summary, better than predicted study efficiency will allow us to recruit more patients in the existing agreed time period. This will account for higher than

expected incomplete data fields, and enhance precision of estimates to make them more clinically useful.

9.3 Statistical Analysis

We will use methods appropriate for small proportions[40] to estimate the prevalence (with 95% confidence interval) of culture positive urines in acutely unwell children under five years presenting in primary care. This will be undertaken on the whole dataset (N= 7000 – 7500). The degree of variation in prevalence between practices and geographical areas will be explored using two level random-effects logistic regression models (with practice/site as a random effect and area as a fixed effect). This analysis will also explore difference by recruitment site type (general practice, WICs, out of hours providers and CEDs). Children in whom urine samples are obtained will be compared to those who are recruited, but no urine sample is obtained in terms of clinical presentation and demographics.

We will compare the probability of contamination in samples that are retrieved via a 'clean catch' method with those using nappy pads, controlling for patient and practice factors in a two level random-effects logistic regression model. We will examine the impact of timing of sample in relation to the time between obtaining the urine transportation (including day of the week) and laboratory analysis on the rates of positive and contaminated urine samples (e.g. exploring if delayed samples such as those taken after daily laboratory collection have an impact on contamination rates).

The sample will then be sub-divided into development and validation datasets. This will be done by randomly selecting practices: all of their patients will then contribute to one of the two datasets. Approximately 4500 – 5000 patients will be used for development of the algorithm and 2000 – 2500, used for validation.

We will develop a clinical prediction rule based on the linear predictor in a logistic regression model in which the outcome variable is a culture-positive urine result. Candidate prognostic variables will be categorised into demographic background and medical history (for example, gender, previous UTI); both specific and general systemic presenting symptoms and signs (for example overall illness severity, fever, vomiting); and results from urine dipstick analysis (nitrite, leukocyte esterase, protein, blood and glucose). Variables will be included in logistic regression models based on an "inclusive" p value threshold of 0.1. We will check for nonlinear effects of continuous variables, and will examine candidate interactions specified *a priori*. Such effects will be included in the final models as necessary. We will begin by examining the predictive value (based on diagnostic odds ratios and C statistics) of the best predictors from each of the three categories

(socio-demographic and previous medical history, clinical assessment, and dipstick urinalysis) of variables. We will then examine the additional diagnostic value of presenting signs and symptoms (compared with socio-demographic and medical history alone) and of dipstick results (compared with the other two categories). We will examine whether it is possible to identify subgroups of children in whom dipstick testing is and is not justified based on their signs and symptoms. The final diagnostic algorithm will be characterised based on its sensitivity and specificity, and positive and negative likelihood ratios.

Diagnostic and prognostic models that are developed using p-value-based variable selection will inevitably suffer from statistical over-optimism. Therefore, the final models will be validated using the second dataset, and the published decision rule will be based on the linear predictors from the model re-estimated in this validation dataset. In the final stages of analysis, we will examine the sensitivity and specificity of the linear predictor, based on a set of chosen thresholds for positivity. A comparison will be made between the results obtained from the validation and the use of shrinkage based approaches applied to the original development dataset.[41] Sensitivity analyses will consider the value of using a different cut-point (e.g. $>10^4$) based on Cardiff NPHS Research Laboratory analysed urine samples and also the impact of making different assumptions relating to contaminated samples.

Children with positive urine cultures ('contaminants' and 'UTIs') who the clinician felt at recruitment had a suspected UTI will be compared to those who the clinician felt there was little probability of a UTI in terms of their subsequent illness course and resource usage over the next three months.

9.4 Minimising Bias

The following design and analytic strategies will be employed to minimise bias:

(1) Selection bias: where possible we will recruit consecutive children; We will ask sites to keep a screening log of patients approached but who did not take part in the study and reasons for this.

(2) Index test technology: all tests (symptoms, signs, nappy pads, dipstick tests) will be carried out using standardised equipment and protocols;

(3) Incorporation bias: the reference standard will consist of culture alone and will not incorporate any of the index tests;

(4) Review bias: observers assessing the index tests will differ from and be blind to those assessing the reference standard (and vice versa);

(5) Verification bias: all children who contribute to the study will have a urine sample sent to assess the reference standard. Children in whom it is not

possible to obtain a sample will be excluded from the analysis. It is unlikely that reasons for failure to obtain urine samples will be related to the index tests but we will compare children with and without urine cultures;

(6) Disease progression bias: we expect the time between clinical assessment and obtaining the urine samples to be minimal;

(7) Treatment paradox: for most children, antibiotic treatment will be started after the urine sample has been obtained, but we will record where this has not been possible;

(8) Handling of indeterminate or uninterruptible results or withdrawals: these parameters will be measured and considered in the analysis, and;

(9) Appropriateness of the reference standard: use of $>10^5$ cfu/ml is likely to detect the majority of children with UTI, but the second 'research' urine (see Economic model structure) will allow for sensitivity analyses around different bacterial concentrations. Where possible, we will measure all threats to validity (e.g. time between clinical assessment and obtaining and culturing the urine sample) that could influence results.

9.5 Sub-group & Interim Analysis

The sample will then be sub-divided into development and validation datasets. This will be done by randomly selecting practices: all of their patients will then contribute to one of the two datasets. Approximately 4500-5000 patients will be used for development of the algorithm and 2000 -2500 used for validation.

We will develop a clinical prediction rule based on the linear predictor in a logistic regression model in which the outcome variable is a culture-positive urine result. Candidate prognostic variables will be categorised into demographic background and medical history (for example, gender, previous UTI); both specific and general systemic presenting symptoms and signs (for example overall illness severity, fever, vomiting); and results from urine dipstick analysis (nitrite, leukocyte esterase, protein, blood and glucose). Variables will be included in logistic regression models based on an "inclusive" p value threshold of 0.1. We will check for nonlinear effects of continuous variables, and will examine candidate interactions specified *a priori*. Such effects will be included in the final models as necessary. We will begin by examining the predictive value (based on diagnostic odds ratios and C statistics) of the best predictors from each of the three categories (socio-demographic and previous medical history, clinical assessment, and dipstick urinalysis) of variables. We will then examine the additional diagnostic value of presenting signs and symptoms (compared with socio-demographic and medical history alone) and of dipstick results (compared with the other two categories). We will examine whether it is possible to identify subgroups of children in whom

dipstick testing is and is not justified based on their signs and symptoms. The final diagnostic algorithm will be characterised based on its sensitivity and specificity, and positive and negative likelihood ratios.

9.6 Cost Effectiveness Analysis

9.6.1 Economic model structure

Cost per correct diagnosis of UTI will be estimated directly from primary DUTY data. However, a decision analysis model is needed to estimate more meaningful long-term cost and outcomes. The model will combine primary data collected in the DUTY study (e.g. accuracy of diagnostic algorithms, duration of symptoms) with literature estimates of unobserved parameters (e.g. effectiveness of antibiotics, incidence of long term complications) to identify the most cost-effective method for identifying children in whom urine collection and laboratory testing is appropriate. The model will also provide a template to measure the cost-effectiveness of 'clean-catch' versus nappy pads for obtaining samples in young children. In a sub-sample of children, we will use time-motion techniques to measure the additional time (parent/guardian and professional) taken to collect the urine sample during the primary care appointment and perform dipstick testing.

A Markov model will estimate the short- and long-run cost-effectiveness of diagnostic algorithms identified in DUTY. This work will build on the cost-effectiveness model developed in our recent HTA report (page 111).[19] The final model structure will depend on the algorithms identified in the primary analysis and discussion with clinicians. New diagnostic algorithms will be compared to two 'boundary strategies': (1) no sample or test - treat all, and (2) no sample or test – treat none. These boundary strategies are not intended to reflect clinical reality, but provide a reference point against which other diagnostic strategies can be compared. In addition, we will include a third, 'clinical judgement' strategy as recorded in the CRF. Analysis of primary data will likely result in a 'high sensitivity' algorithm (i.e. liberal use of urine testing) and a 'high specificity' algorithm (i.e. parsimonious use) that will be compared to the boundary and clinical judgement strategies.

A diagnostic algorithm could assign a child to the high, medium, or low risk of UTI categories. The high-risk group are treated with antibiotics and for those meeting NICE guideline criteria,[20] referred for further investigation (e.g. ultrasound, MCUG or DMSA). Some children are false positives, receiving antibiotics despite not having UTI. Children in the medium risk group have a urine sample sent for laboratory culture. Those who do not have UTI will incur the unnecessary cost of

urine collection and testing. However, assuming that laboratory culture is a gold standard test for UTI, these false positives will be identified before treatment commences. The low risk group will not be tested or treated for UTI, some false negatives may have longer symptom duration, higher risk of recurrence and long-term complications. The long-run Markov model will be based on the HTA model (page 113)[19] allowing for recurrent UTIs with increasing risk of pyelonephritic attack and progressive renal scarring.

Several key model parameters will be informed directly by the DUTY study results. These include the initial prevalence of UTI, the diagnostic accuracy of diagnostic algorithms for identifying children with UTI, the cost of urine collection and the disutility of UTI symptoms. Based on these we will calculate the incremental cost per correct UTI diagnosis of each diagnostic strategy. Symptom duration and acute care costs will also be collected in DUTY; however other parameters, needed to calculate cost per symptomatic day avoided and cost per QALY, will be derived from previous research. The unit costs of tests, imaging, and therapy for UTI and long term complications of UTI will be based on national data (e.g. BNF, PSSRU, NHS reference costs) and published costing studies. Outcome parameters, such as the effectiveness of antibiotics, probability of UTI recurrence, renal scarring, end stage renal disease and survival will be based on literature estimates.[19] Some literature estimates, particularly utility values in young children with UTI, are imprecise or even non-existent. Previous work in UTI[19] used adult utility values as a proxy for young children. Despite imperfect data, we believe that it is important to estimate cost per QALY and use threshold analyses to explore the relationship between the short and long-term disutility caused by UTI and the cost-effectiveness of diagnostic algorithms. These analyses will allow tentative conclusions about the overall relative efficiency of each algorithm compared to other healthcare technologies.

Each parameter will be assigned a probability distribution to propagate parameter uncertainty throughout the model in a probabilistic sensitivity analysis. Costs and outcomes occurring after the first year will be discounted at 3.5%.[39] We will use net monetary benefits and cost-effectiveness acceptability curves, at plausible willingness to pay thresholds (e.g. £0 to £50,000 per QALY) to identify the most cost-effective diagnostic strategy at each threshold. We will test the robustness of our results to the model structure using sensitivity analyses with different structural assumptions. Sensitivity analyses will also be used to evaluate the impact of key parameters on results and the influence of various CFU/ml thresholds on the choice of diagnostic algorithm.

10 Data Storage and Retention

All data will be kept for 15 years in line with Bristol and Cardiff Universities' Research Governance Framework Regulations for clinical research. During the study, data will be stored on secure servers as part of the electronic primary care network. This will be archived after publication of results to a secure location at the University of Bristol.

11 Study Closure

Study recruitment will end when the last patient has been followed up or at the request of the Steering Committee. The end of the study will be considered as the date on which the last participant has completed their follow-up assessment (3 months note review in this case).

12 Regulatory Issues

12.1 Ethical Approval

DUTY will be conducted according to the principles of good research practice (including proper and appropriate conduct of research, professional integrity, honesty, statistical methods, use of data, interpretation of data, non plagiarism) and the Research Governance Framework for Health and Social Care.

The study will comply with NHS Ethics Committees and Health and Safety regulations. The storage, analysis and disposal of urine samples will accord with the requirements of the Human Tissue Act. The study will be approved by an appropriate National Research Ethics Service Committee before commencing.

12.2 Consent

Participation in the study will be entirely voluntary with parents/guardians or those legally allowed to consent for children given full information regarding what study participation involves, their right to withdraw and research dissemination plans. Full written consent will be obtained from those legally allowed to consent on children's behalf, and all research staff with participant contact will have passed Criminal Records Bureau checks. Parents or guardians will be made aware that they can decide to withdraw from the study at any time, with no detrimental impact on current and future medical treatment.

12.3 Confidentiality

The Chief Investigators and the research team of DUTY preserve the confidentiality of participants in accordance with the Data Protection Act 1998. All

research data will be handled according to the principles of the Data Protection Act, especially for sensitive, personal data. Data will be anonymised and stored on a password protected computer located in secure University buildings and appropriately backed up. Data transfer across participant organisations will be closely monitored. A Privacy Risk Assessment in each regional centre will proactively identify and ameliorate risks of breaches to confidentiality and clearly designate the named individuals who will be allowed to access identifiable information. All data will be retained for up to 15 years post study closure in line with University of Bristol procedures.

12.4 Indemnity

The University of Bristol has agreed to sponsor this study. The University of Bristol will be responsible for, and administer the financial aspects of the grant.

The University of Bristol has arranged insurance to cover the legal liability of the University as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University and as protocol authors for harm to participants arising from the design of the research.

All applicants and research staff employed on the study will hold honorary contracts with the appropriate Primary and Secondary Care Trusts, conferring the protection of the NHS clinical negligence arrangements for staff.

All participants will be recruited at NHS sites and therefore the NHS indemnity scheme/NHS professional indemnity will apply with respect to claims arising from harm to participants at site management organisations.

12.5 Study Sponsorship

The University of Bristol will act as sponsor for trial. Delegated responsibilities will be assigned to the Universities and NHS trusts taking part in this study.

12.6 Audits and Inspections

The DUTY trial is participant to inspection by NIHR HTA as the funding organisation. The study may also be participant to inspection and audit by the University of Bristol under their remit as sponsor.

13 Study Management

DUTY will be led by Co-Chief Investigators (CIs) as this ensures optimal integration of two centres that each bring unique expertise necessary for successful achievement of study objectives. For example, Cardiff brings experience from the EURICA study that informs the DUTY study design, recruitment and data collection. Bristol brings world-class expertise on decision tool development. This is a collaboration between four Research Sites that is necessary to ensure sufficient population base to recruit to target, and two trial units (BRTC and the South East Wales Trials Unit (SEWTU)). Each centre will take responsibility for regional recruitment and liaison with local laboratories. BRTC will lead analysis of diagnostic and economic data. SEWTU will lead study set up and the overall data management including design of data collection and entry tools, monitoring data quality, and liaison with the research laboratory. London will lead the design, implementation and maintenance of the web based data collection system.

- Weekly project team meetings involving the CIs and the directly employed staff in each of the two trials units will cover day to day issues on each site.
- Monthly Study Management Group (SMG) meetings consisting of all co-applicants and directly employed staff will oversee the study. These will be predominantly via audio conference, but with an initial face-to face meeting and then face-to-face at least every 6 months. This mix of face-to-face and frequent audio conferences has been highly successful on other collaborations between these teams. Additional meetings will be held on specific topics during the set-up phase covering data management and RN/CSO training.
- An Independent Study Steering Committee (SSC) will be convened to give independent oversight and will consist of a general paediatrician, a general practitioner, a statistician and two lay advisors. This group will meet before recruitment starts to agree the final protocol and then at least annually throughout the study. As this is an observational study with no randomisation or experimental interventions, a separate Data Monitoring and Ethics Committee will not be appointed. Instead, the SSC will take on data monitoring functions.

14 Data Handling and Record Keeping

14.1 Database development and hosting

Data collection and management for DUTY will be conducted using a specific web 2.0 clinical study management system (The electronic Primary Care Research Network (ePCRN)). This secure web-based system is a sophisticated electronic

architecture, developed by King's College London using industry best practice and in collaboration with the NHS Research Capability Programme and the NIHR Primary Care Research Network, to provide research tools to enhance recruitment to NIHR portfolio studies in Primary Care.

Hosted by SLAM NHS Foundation Trust, the ePCRN implementation is of a separate domain and a Citrix farm serving published applications, with an SQL server providing clinical based study application databases, that supports the running and provision of these applications in primary care clinical practices throughout the country. The system avoids potential data loss, duplication and security issues with laptops and portable media and has been approved by ethics and by the SLAM Caldicott and Executive Committees.

The DUTY database will be specifically developed and programmed for this environment in ASP.net, and installed and configured on the ePCRN by King's College London.

Web-forms for data collection will be created in ASP.net on top of a dedicated SQL data management server, with data variables forced to comply with entry and validation rules defined in the data element definitions. The SQL data management server will incorporate auditing, backup and recovery facilities. The study workflow and algorithms will be enforced using the same methods, and a visual algorithm on the web pages will guide users. The web-based system will be piloted for ease of use prior to data entry go-live.

14.2 Electronic data protection and confidentiality

The ePCRN safeguards the legal and ethic rights of service users through a fully integrated research security management system consisting of two component parts: (1) technical specifications built into the DUTY study database during the development phase (see appendix, ePCRN Security Framework Agreement), and (2) procedural standards governing the launch and day-to-day use of the application by DUTY study researchers.

Access to users will be provided through study-specific logon points in Citrix Access Gateway Advanced Access Control. Citrix software establishes a secure, encrypted, connection with the user's PC, allowing access from the internet uniquely to the Citrix Access Gateway and enabling access to identifiable study data for authorized users.

Unique study idenification numbers will be sequentially generated and used on pre-printed consent forms, paper CRFs, urine sample labels and test request forms (both for local NHS and Cardiff research laboratories).

14.3 Data entry in primary care sites

In order to maximise the acceptability and ease of use of DUTY data collection tools, clinicians working in settings without web access or whose working practice made web data entry an unwelcome burden, will be able to opt for paper-based data collection with the support of the local study centre in entering data, or making alternative arrangements for data entry, on their behalf.

The web-based data collection system will be presented as the preferred method of data collection, and practice-based recruiting staff will be strongly encouraged and supported to enter CRF data onto the database directly or, if using paper-based CRFs in the recruitment interviews, to retrospectively enter the data in a timely way (consent and registration within 24 hours, and full eCRF data within 5 working days).

Signed individual consent forms will be obtained prior to patient registration and data entry on the DUTY database.

The unique study number will be entered onto the system by the RN or CSO from the primary care site. It will be possible for the child's socio-demographic, symptoms and signs data to be entered prior, and by a different user, to the urine dipstick results, since these are sometimes only available several hours after recruitment. Data will be entered by the RN/CSO using the ePCRN via the Citrix Access Gateway. All urine specimens received at the site will be logged on the system using a check-box and by entering urine dipstick results.

14.4 Data entry in the local NHS laboratory and Cardiff research laboratory

Once in the local NHS and Cardiff research laboratories, staff will be able to access an anonymised data collection page, where only study numbers and the data collection forms for the urine samples can be seen. Laboratory staff will be asked to log the samples on receipt and enter the results when available.

14.5 Follow-up data entry in study centres

Between Day 12 and Day 21 from recruitment, and at 3 months, research staff will enter cost, symptom duration and health utilisation data, from telephone interviews and/or postal questionnaires, and practice records respectively onto similar web-based data collection forms, based on the study number and demographic details recorded for consenting patients.

14.6 Data management

A full workflow protocol for data management will be developed between Cardiff and King's College London, including access, data element definitions, analysis plans and rules for the closure of the database.

14.7 Recruitment and sample monitoring

The timeline will be enforced at specific workflow check-points where lists of subjects not having results in the required time, or requiring follow up by the clinical site will be automatically generated. Regional staff will access the system each day to identify tasks required.

14.8 Study monitoring

Paper records will be held for consent forms, a copy of which will be filed in the Bristol study office. As a source record of study entry, the facility to generate a read-coded and text entry in the medical record will be provided for pasting into the electronic health record at the time of entry to the study, containing a summary of the baseline data collection and confirmation of consent. Practices will scan the signed consent form into their record.

15 Service User Involvement

15.1 Aims

Parent/guardian collaboration, particularly in the collection of urine samples, is key to the success of this study. The aims of service user involvement will be to: (1) optimise the recruitment strategy; (2) ensure study information and data collection forms are structured (e.g. content, layout, frequency) to optimise completion and minimise data loss; (3) advise on the logistics of urine sample collection, and (4) support the dissemination of results especially to harder to reach groups.

15.2 Methods

This study is heavily informed by the EURICA study that has in effect piloted many of the methods for engaging professionals and parents/guardians. In a pilot study for EURICA, we spoke to over 100 parents//guardians and asked them about their experiences of the study and how this could be improved, which has informed DUTY processes. There will be four levels of parent /guardian involvement in DUTY, and all will have children who currently meet or have recently met (within 3 years) the eligibility criteria: (1) parents/guardians will be involved in further refining the methods and tools for the study through small group (similar to focus group format); (2) parents/guardians invited to participate will be asked for feedback using a 'review of study' questionnaire for voluntary completion at end of active study involvement and;(3) two parents/guardians will be full members of the SSG

(as opposed to the day-to-day management team) to provide feedback throughout the study and help with strategic decisions.

15.3 Dissemination to Participants

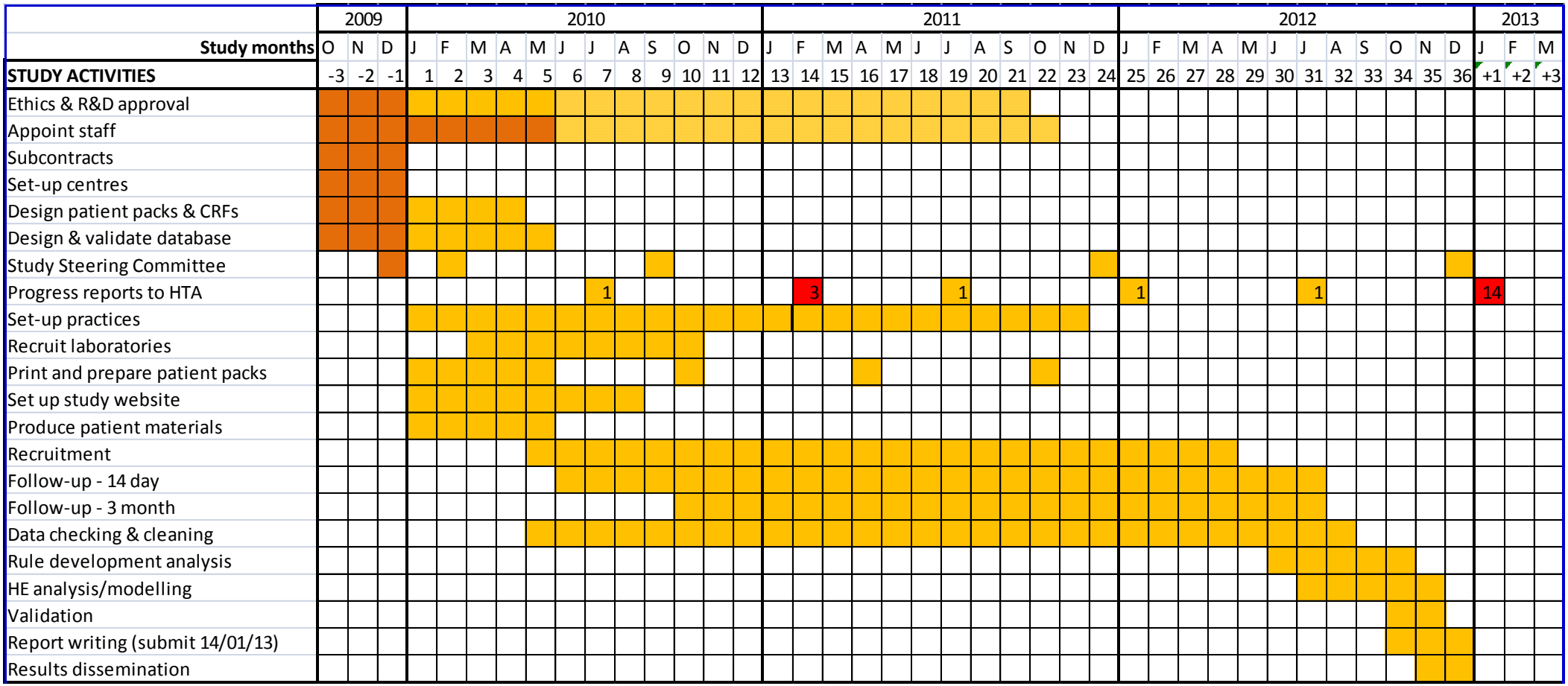
Parents/guardians will be asked to identify how we can best feed back the results to their social groups. At this stage (subject to review towards the end of the study) this is expected to take the form of equipping parent/guardian champions to be able to give a verbal report to local social groups as well as seeking their advice on parent/guardians study results' summaries.

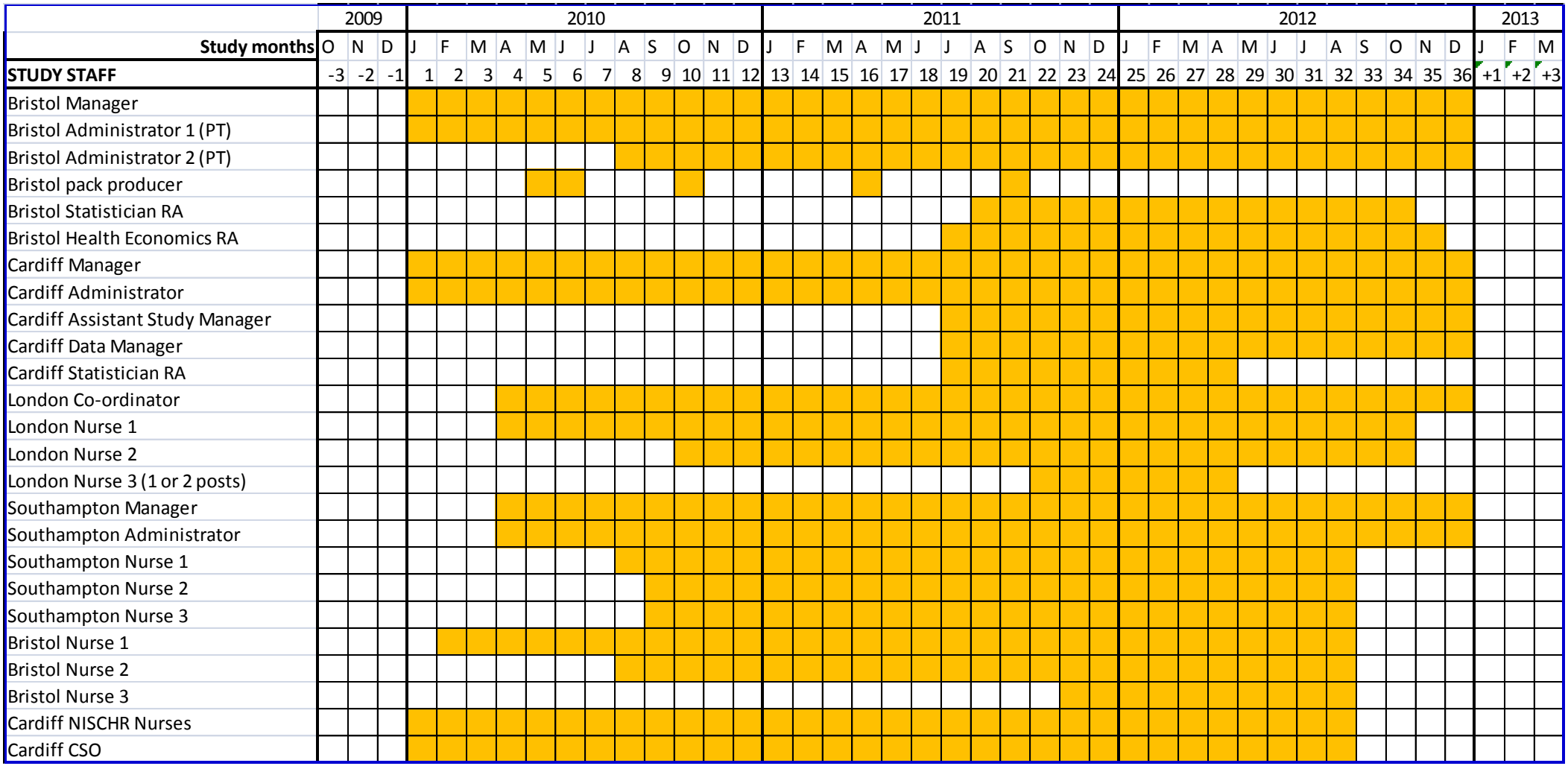
16 Publication Policy

In addition to the required final report and monograph for the HTA Programme, we will publish the main study results in international peer-reviewed journals and present at national and international scientific meetings. With the assistance of our collaborators and service users we will disseminate the study findings to a wide NHS and general audience. This will include presentations at meetings and written executive summaries for key stakeholder groups such as Primary and Secondary Care Trusts, Walk-in Centres, Out of Hours Centres, NHS Direct, health visitor groups, general practices and service users.

17 Project Timetable and Milestones

The Gantt chart below details the main project milestones




2009
2010
2011
2012
2013
Study months
STUDY STAFF
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M
-3 -2 -1 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 +1 +2 +3
Bristol Manager
Bristol Administrator 1 (PT)
Bristol Administrator 2 (PT)
Bristol pack producer
Bristol Statistician RA
Bristol Health Economics RA
Cardiff Manager
Cardiff Administrator
Cardiff Assistant Study Manager
Cardiff Data Manager
Cardiff Statistician RA
London Co-ordinator
London Nurse 1
London Nurse 2
London Nurse 3 (1 or 2 posts)
Southampton Manager
Southampton Administrator
Southampton Nurse 1
Southampton Nurse 2
Southampton Nurse 3
Bristol Nurse 1
Bristol Nurse 2
Bristol Nurse 3
Cardiff NISCHR Nurses
Cardiff CSO

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19 Appendices

Participant information sheets and consent forms, and GP letters, are stored separately.