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DUTY: Diagnosis of Urinary Tract Infections in Young Children Study

Laboratory Manual

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1 INTRODUCTION

This manual is intended to provide the staff of microbiology laboratories participating in the DUTY study with information about their role in the study, study procedures and materials.

The manual provides study information for the local NHS microbiology laboratories, and the central research laboratory in Cardiff (SACU), participating in the DUTY study.

For NHS laboratories participating in DUTY, the Study team has provided:

- This DUTY Study - Laboratory Manual
- Study Patient Identifier Labels (pre-printed with unique ID numbers)
- Instructions for Data Entry onto DUTY Website

For the Central Research (SACU) laboratory, the DUTY Study team has provided the above, and in addition:

- A Central Research Laboratory Requisition Form

1.1 Sampling materials

For NHS Local Lab urine sample:

- Usual local NHS urine sample container or (depending on local laboratory's normal practice for very small samples)
- Sterilin container (provided by DUTY team)

For Central Research Lab (SACU) urine sample:

- Urine Monovette with Boric Acid Stabiliser
- Sample Transport Tube
- Royal Mail 'Safebox'

1.2 Documents

The sampling materials above are accompanied by a **Requisition Form** and identifying DUTY labels.

For NHS Local Lab urine sample:

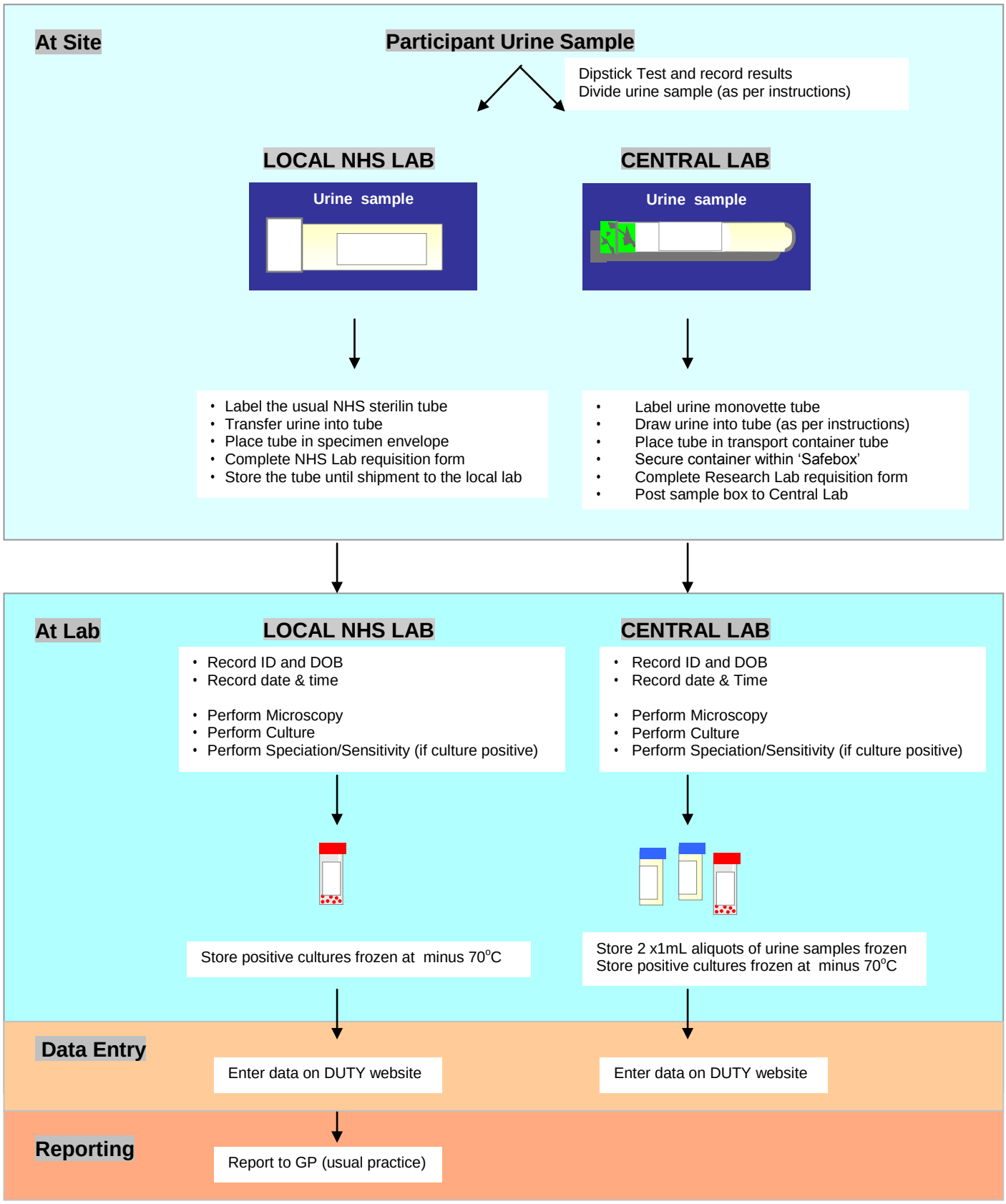
- The **DUTY Research Nurse/Clinical Studies Officer (RN/CSO)** will complete the normal **NHS Laboratory Requisition Form**, label the sample container and the requisition form with the study-specific labels, and follow local procedures for sample processing.

For Central Cardiff (SACU) Lab urine sample:

- In addition, the **RN/CSO** will complete the **Central Research Laboratory Requisition Form**, and attach a DUTY label, for all samples sent to Central Research Laboratory (SACU). These research samples and forms will be transported in Royal Mail 'Safebox' containers.

2 SAMPLE HANDLING PROCEDURE

2.1 Sample Handling Overview



2.2 Sample Processing Overview

The DUTY team researcher (Research Nurse/Clinical studies Officer [RN/CSO]) responsible for the urine sample should, once a sample has been obtained, follow the procedure described below:

Before splitting urine

- Perform urine dipstick test before separating urine and record results on CRF
- Record date and time of urine dipstick testing on CRF

Urine splitting procedure

- Ensure following are to hand:
  1. Usual NHS container or Sterilin Universal container (depending on local laboratory's standard practice for very small samples)
  2. Four sets of DUTY patient ID labels (for urine sample containers AND requisition forms for local and Central Research laboratories)
  3. Pasteur pipette
  4. Urine monovette® (with boric acid) and plastic tip
  5. Urine monovette® transport container and lid
  6. Dedicated mailing box for Urine monovette®

2.2.1 Local NHS Laboratory Sample (PRIORITY fraction):

Please note: A minimum of 2mL is required for the local laboratory.

- Label NHS sample container with DUTY label (with pre-printed Patient ID number).
- Using a plastic Pasteur pipette, transfer 2 ml urine to sample container.
- Complete NHS Local Laboratory requisition form as per usual practice, with the following:
  - Practice information (Site ID number, Practice Name, Street Address, Postcode & Contact telephone number)
  - Patient gender (F/M)
  - Date of Birth (dd/mm/yyyy)
  - Urine Collection Date (dd/mm/yyyy)
  - Urine Collection Time (24 hours clock)
  - **Attach DUTY label** (with Patient ID number)

2.2.2 Central Research Laboratory Sample (RESEARCH fraction)

Please note: A minimum of 2mL is required for the Central research Laboratory. If remaining volume of urine is < 2mL, please send all remaining sample. If remaining volume is >2mL, please fill monovette®.

- Label empty Urine monovette® with DUTY sticker (with pre-printed Patient ID number).
- Gently remove the small stopper from the Urine monovette® and attach plastic tip.
- Insert the plastic straw of the Urine monovette® into the remaining urine sample.

- Gently pull out the plunger of the Urine monovette® and fill it with urine.
- To empty the plastic tip, hold the Urine monovette® with the straw tip facing upwards and pull the plunger backwards fully to the bottom of the tube.
- Remove the plastic tip, break off the plunger and discard it.
- Replace the stopper.
- Mix the sample by inverting the Urine monovette® several times, ensuring that the boric acid is completely dissolved.
- Place the Urine monovette® in the mailing container tube with the stopper end at the top.
- Close with the screw-top and place in mailing box for transport to Central Research laboratory using DUTY address label.
- Complete DUTY Research Lab specific requisition form, and label with a DUTY sticker (as described below).

2.2.3 Central Research Lab Requisition Form

Complete the following information on the DUTY Research Laboratory Requisition Form (Annex 1):

- Practice information (Site ID number, Practice Name, Street Address, Postcode & Contact telephone number)
- Patient gender (F/M)
- Date of Birth (dd/mm/yyyy)
- Urine Collection Date (dd/mm/yyyy)
- Urine Collection Time (24 hours clock)
- **Attach DUTY label** (with Patient ID number)

3 Laboratory Urine Analysis

3.1 Local NHS Laboratory

3.1.1 Sample Identification

Each tube should be identified with a DUTY study label. Labels are coded with a patient study number (a unique ID number). The sample label should match that on the requisition form.

3.1.2 Arrival of Samples

At the moment of the arrival of the samples in the local lab, the following information should be recorded, and the required fields on the DUTY Study website (www.dutystudy.org.uk) completed using your laboratory's unique user ID and password (to be provided by the study team).

- Local Laboratory study number (Lab ID)
- Patient study number
- Arrival Date of the urine sample (dd/mm/yyyy)
- Arrival Time of the urine sample (24 hours clock)

Check the labels on the specimen match the label on the Local NHS Laboratory Requisition Form. The label information should be identical. If not, please contact the local DUTY Study team *(see contact details at the end of this document).*

3.1.3 Urine Analysis (as per local SOP)

In the Local NHS Laboratory the following tests are to be performed in accordance with local standard operating procedures (DUTY Study team should have a copy):

- Microscopy (if available)
- Culture
- Speciation/Sensitivity

If urine cultures are positive for UTI (>10^5CFU/mL), prepare positive cultures samples for frozen storage.

After preparation of positive culture, label the samples with patient ID number and DOB.
Store them in your freezer, preferably at minus 70°C until shipment to the Central Lab.

If the facilities to freeze at minus 70°C are unavailable to you, then the samples can be stored at minus 20°C.

3.2 Central Research Laboratory (SACU)

3.2.1 Sample Identification

Each tube should be identified with a label. Labels are coded with a patient study number (a unique ID number). The sample label should match that on the requisition form.

3.2.2 Arrival of samples

At the moment of the arrival of the samples in the local lab, the following information should be recorded, and the required fields on the DUTY Study website (www.dutystudy.org.uk) completed using your laboratory's unique user ID and password (to be provided by the study team).

- Local Laboratory study number (Lab ID)
- Patient study number
- Arrival Date of the urine sample (dd/mm/yyyy)
- Arrival Time of the urine sample (24 hours clock)

Check the labels on the specimen match the label on the Research Lab Laboratory Requisition Form. The label information should be identical. If not, please contact the local DUTY Study team *(see contact details at the end of this document).*

3.2.3 Urinalysis as per SACU SOP (see appendix 4)

a) Processing of urine:
    a) Microscopy
    b) Presence of anti-microbial substances
    c) Culture (by spiral plating)
    d) Isolate identification
    e) Susceptibility testing

If cultures are positive for a single organism > 10^3CFU/mL:

- Store 2 x1mL aliquots of urine sample at minus 70°C.
- Prepare culture for storage at minus 70°C.

4 FROZEN SAMPLE SHIPMENT PROCEDURE

The local study team will liaise with the NHS laboratories about transporting stored samples to the Central Research Laboratory in Cardiff either at the end of the study or if more convenient in batches at intervals to be agreed.

5 REPORTING

5.1 Local NHS Laboratory

The NHS Local Lab will report the following data into the DUTY study web database (www.dutystudy.org.uk):

- Arrival date (dd/mm/yyyy) and time of the urine sample (24 hours clock)
- Date of sample processing (urinalysis)
- Results of microscopy for urine sample (if available)
- Results of urine culture (as detailed in table)
- Results of Speciation/Sensitivity
- Date and time of results reported to GP

Data should be entered within **5 working days** after the samples are received at the lab. Priority should be given to entering data for culture positive samples as close to real time as possible, to facilitate patient follow-up by the study team.

(see data tables in Appendix 2)

5.2 Central Research Laboratory

The Central Lab will enter the following data into the DUTY study website:

- Arrival date (dd/mm/yyyy) and time of the urine sample (24 hours clock)
- Date of sample processing (urinalysis)
- Results of microscopy for urine
- Results of Culture (as detailed in table below)
- Results of Speciation/Sensitivity

Data should be entered within **5 working days** after receiving of the samples at lab.

(see data tables in Appendix 3)

5.3 DUTY Study Team

Reporting on discrepant results:

- Collate results.
- If NHS lab result is negative and Research Lab result is positive for UTI, inform responsible clinician and local NHS Laboratory Manager (or other lead contact as identified by the laboratory) of discrepant result.

6 DUTY STUDY CONTACTS

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7 APPENDICES

7.1 Appendix 1: DUTY Central Research Lab Requisition Form

DUTY RESEARCH LAB REQUISITION FORM

Recruitment Site number:
Recruitment Site Name:
Street Address:
Postcode:
Country:
Telephone:

PID UNIQUE NUMBER:
(*use DUTY label*)

PATIENT GENDER

F/M

DATE OF BIRTH

d d m m y y y y

COLLECTION DATE

d d m m y y y y

URINE COLLECTIONTIME

h h : m m

INSTRUCTIONS:
After urine collection, label the monovette tube with a DUTY sticker. Place the tube in the transport container. Secure the container within the Safebox postage box provided, together with this **DUTY Requisition Form**. Store the sample in the refrigerator until posted to the Central Research Laboratory.

Please make sure that the Lab Requisition Form is filled in completely and legibly. If an error is made, cross out the mistake, write the correct information and initial and date the change.

DUTY WEBSITE: http://www.dutystudy.org.uk

7.2 Appendix 2: DUTY Local NHS Data Entry Tables

Microscopy

Microscopy performed: Yes/No						
If Yes indicate method:	Manual				Automated	
	Count (mm^3)					
Microscopy Results	WBC	<10	10-30	30-100	>100	Absolute WBC count
	RBC	0	<5	6-100	>100	
	Squamous Epithelial Cells	0-5	6-75	>75		

Culture

Culture performed: Yes/No If No: please give reason (*free text*)

i) Growth

Growth: (please indicate)	No growth	No significant growth	
	$\leq 10^3$ CFU/mL	10^3-10^5 CFU/mL	$>10^5$ CFU/mL

Purity: (please indicate)	Pure/Predominant	Mixed growth (2 species)	Mixed growth (> 2 species)
Speciation: (please indicate)	For organism 1 (see below)	For organism 1 & 2 (see below)	Not Applicable
Sensitivity: (please indicate)	For organism 1 (see below)	For organism 1 & 2 (see below)	Not Applicable

ii) Speciation and Sensitivity (for each organism identified from above)

Organism 1 (complete table for each identified) Drop down box:	**Speciation** **Basic ID** Coliform Proteus** Enterococcus Other	**Additional ID** (***if available***)	**Sensitivity** Menu of antibiotics tested	**Results** (record for each item tested)		
				S	I	R

(** Proteus is classified as a coliform, but recorded separately)

7.3 Appendix 3: DUTY Central Research Laboratory Data Entry Tables

Microscopy

Microscopy Method	Manual	Automated	Not Measured
	Count (mm^3)		
WBC	Absolute WBC count		
RBC	Absolute RBC count		
Squamous Epithelial Cells	Absolute SEC count		

Culture

i) Growth

Growth: (please indicate)	No growth	No significant growth
	Enter TOTAL count here (CFU/L)	

Purity: (please indicate)	Pure/Predominant	Mixed growth (2 species)	Mixed growth (> 2 species)
Speciation: (please indicate)	For organism 1 (see below)	For organism 1 & 2 (see below)	Not Applicable
Sensitivity: (please indicate)	For organism 1 (see below)	For organism 1 & 2 (see below)	Not Applicable

ii) Speciation and Sensitivity (for each organism identified from above)

Organism 1	**Colony Count (CFU/mL) (enter absolute count)**				
Organism 1 (complete table for each identified)	**Speciation**	**Sensitivity**	**Results** (record for each item tested)		
Drop down box:	*E.coli*	Menu of antibiotics tested	S	I	R
	Enterococci				
	Klebsiella-Enteobactor-Serriata group				
	Proteus				
	Staphlococcus saprophyticus				
	Pseudomonas aeriginosa				
	Other Staphylococci/Streptococci				
	Candida				
	Morganella-Providencia group				
	Other organisms: (please indicate)				

Please enter any other Information here	*Free Text*

iii) Detection of Antimicrobial Substances

Presence of Antimicrobial Substances	Yes/No