FIEBRE Standard Operating Procedure F.08c			
Title	**Urine Dipstick Use, and Culture Preparation, Interpretation, and Results Recording**		
SOP Reference		***Version***	***Date of effect***
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SOP Development

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Revision History

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1.1.2	18 Dec 2018	Minor formatting updates

SOP User Confirmation

I acknowledge that I have read, understood and agree to follow this SOP

#	Name (print)	Signature	Date
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1 Title: URINE DIPSTICK USE, AND CULTURE PREPARATION, INTERPRETATION, AND RESULTS RECORDING

2 Purpose: To describe the procedures for dipstick testing of FIEBRE urine samples; and for isolating, quantifying and permitting presumptive identification and differentiation of the major micro-organisms causing urinary tract infections (UTIs) for FIEBRE study participants.

3 Responsible staff: FIEBRE microbiology laboratory staff

4 Background & Rationale: UTIs are relatively common, especially in young children and in adult women, and may be a cause of fever. Detection of these infections is important both for good clinical care and for the FIEBRE study.

This SOP describes general procedures for processing and storing urine culture samples obtained as part of the FIEBRE study. For specific details on individual steps, please refer to your laboratory's accepted SOPs, manuals and procedures. Feel free to ask the FIEBRE study co-ordinator and your laboratory supervisor for guidance whenever needed.

For FIEBRE study patients, urine samples will be obtained from all young children (aged <2 years), and from older patients who have symptoms and signs of UTI. We will first test each urine sample with a standard dipstick test. If the dipstick is positive for nitrites and/or leukocyte esterase, we will culture the urine sample. [Urine samples will also be obtained for lipoarabinomannan testing where able in adult participants in areas where HIV prevalence is >/= 1% in the general adult population - for processing of these samples see SOP F-06c].

Since voided urine is not sterile, urine cultures are typically prepared using a "semi-quantitative" method: A known volume of urine is cultured in order to allow quantification of the number of organisms in the original urine.

The medium used to culture organisms from urine, Oxoid *Brilliance*™ UTI Clarity™ agar, contains two specific chromogenic substrates. The different reactions produced by the micro-organisms, if present, leads to different isolates appearing as different coloured colonies after overnight incubation at 37°C in air.

Ideally urine microscopy and culture should be performed on a mid-stream or “clean catch” urine sample. However, due to difficulty in obtaining this type of sample, especially in children or catheterized patients, occasionally “bag urine” or catheter samples will be received; the results of such samples require careful interpretation (as described below).

For culture-positive samples from FIEBRE patients, cryopreserved isolates will be shipped on dry ice to an international reference laboratory for confirmation of identification and of antimicrobial susceptibility.

5 Supplies and Materials

- Sample logbook (paper or Open Data Kit [ODK])
- Urine culture logbook or records (paper or ODK)
- Participant’s urine sample in sterile container
- Sample labels with patient’s QR or other barcode
- Gloves (single-use latex or vinyl)
- Sterile 1 µL and 10 µL loops
- Oxoid Brilliance™ UTI Clarity™ agar (chromogenic agar plate)
- **For a complete list of materials and reagents needed for each Procedure mentioned in this FIEBRE study SOP, refer to your lab’s local SOPs**
- Biohazard disposal system

6 Procedures:

6.1 Timing of the urine sample

Note the time the urine sample was taken and the time of receipt in the laboratory. If there is a delay of more than 2 hours and the sample has not been refrigerated, the sample is unsuitable for processing and should be discarded, and a repeat sample must be requested. If there will be a delay in culturing a urine sample, it should be refrigerated.

6.2 Performing a urine dipstick

Always wear latex or vinyl gloves when handling urine culture specimens, to prevent exposure to pathogens. Observe standard universal precautions when obtaining and handling urine cultures.

6.2.1 Turn the urine container, with the lid tightly closed, over three times to mix it carefully. Then, with the container upright, remove the lid.

6.2.2 Follow manufacturer's instructions for preparation of the urine dipstick; see package insert.

6.2.3 If the dipstick is positive for nitrite and/or for leukocyte esterase, prepare a urine culture as described below. If the dipstick is negative for both nitrite and leukocyte esterase, record the negative result in [ODK/paper logbook], and discard the urine sample according to local standard practice.

6.2.4 If the dipstick is positive for any other analyte (glucose, haemoglobin, etc], report the result to the patient's clinical team for appropriate follow-up.

6.3 Preparing urine cultures

6.3.1 Turn the urine container, with the lid tightly closed, over three times to mix it carefully. Then with the container upright, remove the lid.

6.3.2 Dip the end of a 1 µL loop into the urine and remove it vertically, making sure that there is no urine on the shaft of the loop (as this would increase the volume transferred for culture).

6.3.3 Spread the entire volume of urine over the surface of a Brilliance UTI Clarity agar plate by making a single streak across the centre,

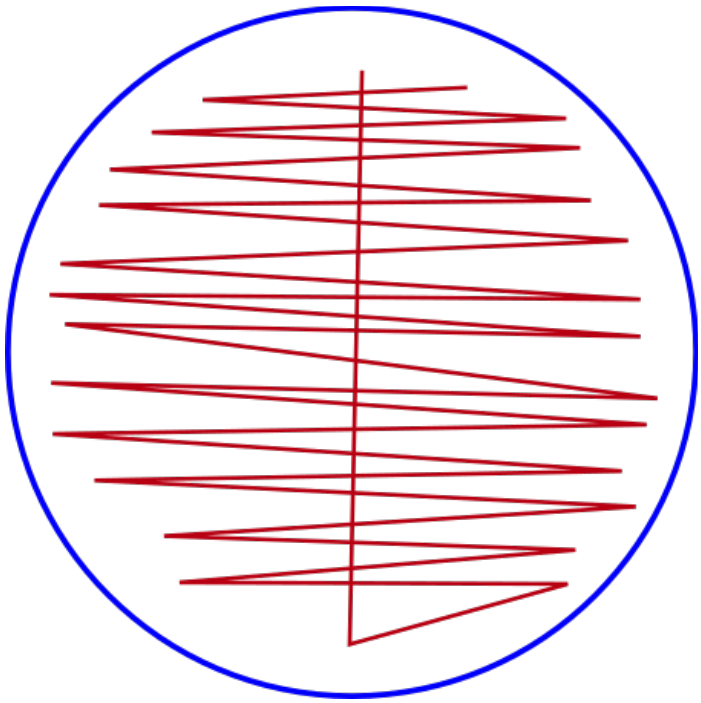


Figure A: Urine streak method

then spreading the inoculum evenly at right angles to the primary streak.

6.3.4 Discard the remaining urine according to local standard practice.

6.3.5 Incubate the chromogenic agar plate aerobically at 35-37° C for 18-24 hours.

6.3.6 After incubation, estimate the number of bacteria on the agar by counting the number of colonies on the surface of the medium. One colony = 1000 cfu/mL (colony forming unit per millilitre).

6.4 Interpretation of urine culture results

6.4.1 Culture results are categorised by quantity and purity of growth. Below a recognised threshold (10^5 cfu/mL), it is likely that the organisms grown are contaminants, particularly if more than one type of organism is present. Above this threshold, it is more likely that a true urinary infection is occurring.

Table 1. Number of colonies on plate

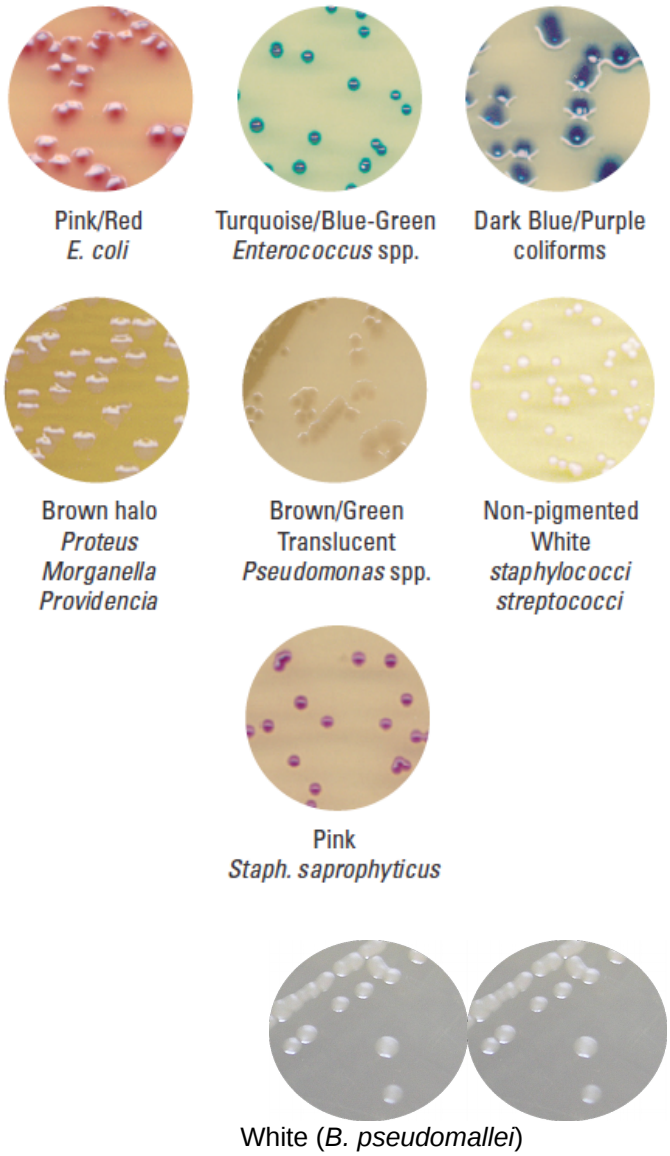
Number of colonies in 1mL urine (on agar plate)	Interpretation	Report
1 – 9	$<10^4$ cfu/mL	No significant growth
10 – 99	10^4-10^5 cfu/mL	10^4-10^5 cfu/mL
≥100	$\geq 10^5$ cfu/mL	$\geq 10^5$ cfu/mL

6.4.2 On day one, if there is a pure growth of 10-99 or ≥100 colonies, identify the organism/s as in Table 2. If 10-99 or ≥100 colonies of two organisms are seen, sub-culture the isolates, and then identify as in Table 2. For cultures that contain two organisms, one in low numbers (<100 colonies) and the other ≥100 colonies, only sub-culture the predominant organism (because the organism present in lower numbers is unlikely to be causing disease).

6.4.3 For catheter-sampled urine (CSU) and bag urine samples, follow the above procedure. However, any growth from these samples, even when pure, should be considered of doubtful significance.

6.4.4 Identify the colonies by their appearance, using the figure as a guide.

Figure B. Interpretation of colony colour on *Brilliance* UTI Clarity agar



6.5 Confirmatory identification, susceptibility testing and reporting of urine culture results

6.5.1 **According to your laboratory's SOPs**, perform Gram stain and confirmatory identification methods for each organism presumptively identified by colour on *Brilliance* UTI Clarity agar. Record genus and species; where identification is uncertain, record the presumptive genus and species for verification at the FIEBRE microbiology reference laboratory.

6.5.2 **According to your laboratory's SOPs**, perform susceptibility testing for the organisms identified.

6.6 Saving/preserving isolates

6.6.1 For urine isolates, cryopreserve only organisms that are judged to be significant, as described in section 6.4.

6.6.2 **Use your laboratory's usual procedure** for cryopreserving urine isolates, in duplicate.

6.6.3 Label each of the duplicate cryovials with both sample and patient identifiers. Use at least two patient identifiers [standardize labelling per site].

6.6.4 Place one cryovial in a cryobox to be sent to the reference lab, and store the duplicate vial at your study site.

6.6.5 Freeze the vials at -70° C or below.

6.6.6 [Record in the log book/culture logbook; per site.]

6.7 Reporting microbiology results

Report all positive microbiology results to the clinical team and study coordinator in real time, using appropriate local procedures [per site].

7 Documentation:

- FIEBRE protocol (version 2.5, 31 Jul 2018) section 7.6.4
- Local laboratory SOPs for preparation of urine cultures, identification and susceptibility testing of microorganisms, cryopreservation of microorganisms, reporting of microbiology results to clinical teams, and other routine microbiology laboratory procedures