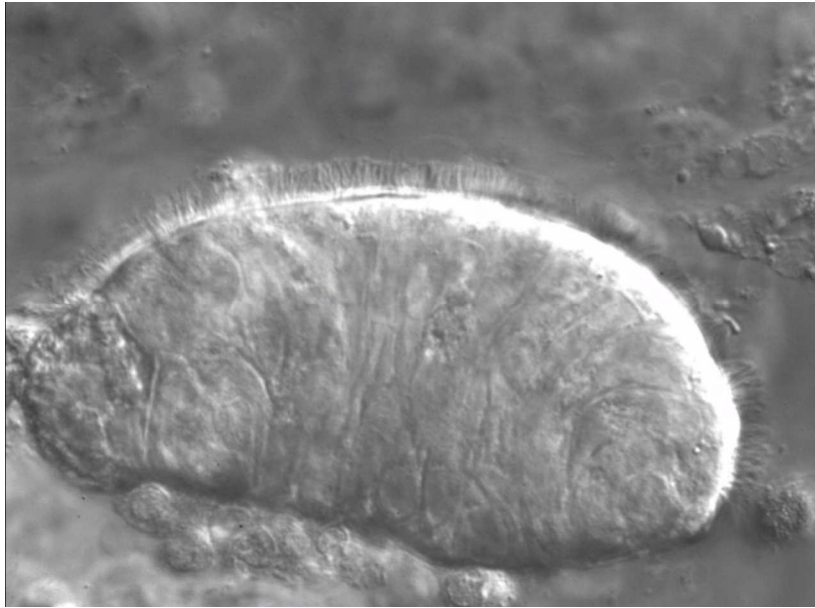


Video 1 Thumbnail



Video 2 Thumbnail

## **SUPPLEMENTAL MATERIAL**

### **Supplemental Methods and Results**

Nasal epithelial tissue samples were obtained using Rhino-Probe curettage of the inferior nasal turbinate. High-speed video microscopy was performed and the tissue placed in culture. Upon culture, nasal epithelial cells lose their cilia, dedifferentiate and undergo mitoses whereupon they were grown to confluence. After confluence was achieved, cells were placed in suspension culture, allowed to reciliate, and video microscopy performed on the reciliated tissue. Ciliary motion (CM) of both the initial nasal scrape as well as reciliated tissue was evaluated by a panel of three investigators (CWL, MZ, OK) blinded to the patient phenotype and nNO values. Five to fifteen videos were reviewed for each sample/subject and CM classified as 1 – normal; 2 – probably normal; 3 – probably abnormal; or 4 – abnormal by consensus evaluation. To create a binary variable, those with scores of 1 or 2 were categorized as normal CM and those with 3 or 4 were categorized as abnormal CM. Samples were not obtained on patients with evidence of ongoing respiratory disease. Whenever reciliation was possible, final determination of CM was made based on findings from reciliated epithelial tissue, to exclude secondary (environmental, medication) causes of abnormal CM. Twenty-seven (43%) of 63 patients had successful reciliation of nasal epithelial cells. Three (11%) patients had a change in the classification of their ciliary motion after reciliation was performed.

Most patients underwent genetic evaluation with a CGH+SNP microarray<sup>1</sup> (constructed by the International Standards for Cytogenomic Arrays), which was performed by the Pittsburgh Cytogenetics Laboratory at Magee-Women's Hospital of University of Pittsburgh Medical Center. In patients with suspected genetic syndromes in whom a microarray was normal, a genetics consultation was obtained to determine need for additional genetic testing. In 8% (5/63) patients, no genetic testing was available for review.

## Supplemental Tables

**Supplemental Table 1.** Clinical characteristics of recruited non-HTX CHD patients

| <b>ID #</b> | <b>Anatomic Description</b>                  | <b>Comorbidities*</b>                                     | <b>Genetics</b>                                      |
|-------------|--|---|--|
| 7004        | DILV, D-TGA, Type A interrupted aortic arch  | Bronchomalacia, seizures                                  | Normal microarray                                    |
| 7027        | D-TGA  | Situs inversus totalis, seizures                          | Normal microarray                                    |
| 7035        | ASD, VSD                                     | Tethered cord, hypothyroidism                             | Multiple congenital anomalies with normal microarray |
| 7040        | ASD, VSD                                     | Bilateral grade I vesicoureteral reflux                   | Normal microarray                                    |
| 7042        | HLHS   |   | Normal microarray                                    |
| 7053        | D-TGA  |   | Normal microarray                                    |
| 7057        | D-TGA  |   | Normal microarray                                    |
| 7058        | TOF  | Tethered cord, exotropia, neurogenic bladder, hydroureter | 8p deletion, 13q12.11 duplication                    |
| 7059        | Variant AVSD                                 |   | Normal microarray                                    |
| 7102        | TOF-PA, MAPCAs                               | Hypocalcemia  | Normal microarray                                    |
| 7113        | DORV, partial AVSD                           |   | Normal microarray                                    |
| 7129        | D-TGA  |   | Normal microarray                                    |
| 7130        | DOLV, malaligned VSD, aortic arch hypoplasia |   | Normal microarray                                    |
| 7134        | DORV, subaortic VSD                          | Aspiration, laryngeal cleft                               | 22q11 deletion (DiGeorge)                            |
| 7146        | HLHS   | Developmental dysplasia of hips                           | 1q.21 duplication                                    |
| 7151        | D-TGA  | Atopic dermatitis   | Normal microarray                                    |
| 7168        | DILV, L-TGA, coarctation                     |   | 3p24.3 deletion                                      |
| 7190        | TOF  |   | Normal microarray                                    |
| 7199        | HLHS   | Hypothyroidism  | 20p11.21 duplication                                 |
| 7208        | D-TGA  | Duplicated renal collecting system                        | Normal microarray                                    |
| 7225        | HLHS   | Hypothyroidism  | Normal microarray                                    |
| 7242        | Truncus arteriosus                           |   | 18p11.32 duplication                                 |
| 7253        | Coarctation, VSD                             |   | No testing available                                 |
| 7266        | Truncus arteriosus                           |   | No testing available                                 |
| 7281        | D-TGA  |   | 10q21.3q22.1 deletion                                |
| 7288        | Truncus arteriosus                           | Hydronephrosis  | Normal microarray                                    |
| 7295        | D-TGA  |   | No testing available                                 |
| 7302        | HLHS   |   | Normal microarray                                    |
| 7306        | D-TGA  | Ectopic kidney  | Normal microarray                                    |
| 7319        | DORV, subaortic VSD                          | Ectopic kidney  | 6p25.1p24.3 duplication                              |
| 7336        | D-TGA  |   | Normal microarray                                    |
| 7351        | HLHS   | Bilateral renal dysplasia                                 | 16p13.3 deletion (Kabuki)                            |
| 7374        | TA, L-TGA                                    | Supraventricular tachycardia                              | Normal microarray                                    |

|             |  |   |                                    |
|-------------|--|---|------------------------------------|
| <b>7376</b> | Truncus arteriosus                     | Micrognathia, subglottic stenosis                                       | 22q11 deletion (DiGeorge)          |
| <b>7379</b> | TOF-absent pulmonary valve             |   | Normal microarray                  |
| <b>7380</b> | Critical aortic stenosis               |   | Normal microarray                  |
| <b>7381</b> | D-TGA                                  |   | Normal microarray                  |
| <b>7386</b> | HLHS                                   |   | Normal microarray                  |
| <b>7389</b> | HLHS                                   |   | Normal microarray                  |
| <b>7397</b> | TOF-PA                                 |   | Normal microarray                  |
| <b>7400</b> | HLHS                                   |   | Normal microarray                  |
| <b>7401</b> | DORV, D-TGA                            |   | Normal microarray                  |
| <b>7405</b> | Infradiaphragmatic TAPVR               |   | Normal microarray                  |
| <b>7409</b> | TA, D-TGA, aortic arch hypoplasia      | Laryngomalacia  | No testing available               |
| <b>7411</b> | Aortic arch hypoplasia                 | Intrauterine growth restriction   | Normal microarray                  |
| <b>7414</b> | Aortic arch hypoplasia, partial AVSD   |   | Normal microarray                  |
| <b>7415</b> | TOF-PA                                 |   | Normal microarray                  |
| <b>7421</b> | TOF                                    |   | Normal microarray                  |
| <b>7422</b> | D-TGA                                  |   | Normal microarray                  |
| <b>7425</b> | Shone complex                          |   | Normal microarray                  |
| <b>7428</b> | Shone complex                          | Supraventricular tachycardia  | Normal microarray                  |
| <b>7433</b> | D-TGA                                  | Prematurity, chronic lung disease, grade II interventricular hemorrhage | Normal microarray                  |
| <b>7437</b> | PA-IVS                                 |   | Normal microarray                  |
| <b>7438</b> | HLHS                                   | Aspiration, hypospadias   | Normal microarray                  |
| <b>7441</b> | DORV, subaortic VSD                    |   | Normal microarray                  |
| <b>7444</b> | DILV, L-TGA                            | Neuroirritability   | 2p16.3 microdeletion (NRNX1)       |
| <b>7447</b> | DILV, straddling tricuspid valve       |   | Normal microarray                  |
| <b>7448</b> | ALCAPA                                 |   | No testing available               |
| <b>7449</b> | DORV, PA, superior-inferior ventricles | Medical necrotizing enterocolitis                                       | Normal microarray                  |
| <b>7450</b> | TOF-PA                                 |   |                                    |
| <b>7458</b> | D-TGA                                  |   | Normal microarray                  |
| <b>7459</b> | Shone complex                          |   | Xp22.33q28 triplication (Triple X) |
| <b>7466</b> | HLHS                                   | Medical necrotizing enterocolitis                                       | Normal microarray                  |

Definition of Abbreviations: DILV = double inlet left ventricle; D-TGA = D-transposition of the great arteries; DORV = double outlet right ventricle; ASD = atrial septal defect; VSD = ventricular septal defect; HLHS = hypoplastic left heart syndrome; TOF = Tetralogy of Fallot; AVSD = atrioventricular septal defect; PA = pulmonary atresia; MAPCAs = multiple aortopulmonary collateral arteries; DOLV = double outlet left ventricle; L-TGA = L-transposition of the great arteries; TAPVR = total anomalous pulmonary venous return; TA = tricuspid atresia; PA-IVS = pulmonary atresia-intact ventricular septum; ALCAPA = anomalous left coronary artery off the pulmonary artery

\*Comorbidities present at the time of patient recruitment

**Supplemental Table 2.** Demographic data for total patients and subcategorized by ciliary motion and nasal nitric oxide\*

|                                  | All Patients<br>(n=63) | Ciliary Motion<br>(n=59 patients) |                    | nNO<br>(n=56 patients)       |               |
|----------------------------------|------------------------|-----------------------------------|--------------------|------------------------------|---------------|
|                                  |                        | Normal<br>(n=40)                  | Abnormal<br>(n=19) | Normal<br>(n=34)             | Low<br>(n=22) |
| Age at Recruitment (days)        | 10 (3–29)              | 9 (3–26)                          | 7 (4–55)           | 5 (3–21)                     | 24 (6–53)     |
|                                  |                        | <i>P</i> = 0.42                   |                    | <i>P</i> = 0.038             |               |
| Male Gender, n (%)               | 42 (67%)               | 27 (68%)                          | 15 (79%)           | 26 (76%)                     | 13 (59%)      |
|                                  |                        | <i>P</i> = 0.55                   |                    | <i>P</i> = 0.28              |               |
| Caucasian Race, n (%)            | 55 (87%)               | 36 (90%)                          | 15 (79%)           | 30 (88%)                     | 20 (91%)      |
|                                  |                        | <i>P</i> = 0.42 <sup>†</sup>      |                    | <i>P</i> >0.99 <sup>†</sup>  |               |
| Gestation >37 weeks, n (%)       | 61 (97%)               | 39 (98%)                          | 19 (100%)          | 34 (100%)                    | 20 (91%)      |
|                                  |                        | <i>P</i> >0.99 <sup>†</sup>       |                    | <i>P</i> = 0.15 <sup>†</sup> |               |
| Normal Genetic Evaluation, n (%) | 45 (76%)               | 32 (80%)                          | 11 (58%)           | 25 (74%)                     | 15 (68%)      |
|                                  |                        | <i>P</i> = 0.3 <sup>†</sup>       |                    | <i>P</i> = 0.33 <sup>†</sup> |               |
| Conotruncal Defects, n (%)       | 36 (57%)               | 19 (48%)                          | 16 (84%)           | 21 (62%)                     | 10 (45%)      |
|                                  |                        | <i>P</i> = 0.010                  |                    | <i>P</i> = 0.36              |               |

\**P* values obtained by Wilcoxon rank-sum test for continuous variables or Pearson  $\chi^2$  test for categorical variables

<sup>†</sup>*P* value by Fisher's exact test

**Supplemental Table 3.** Ciliary motion and nasal nitric oxide values in recruited CHD patients

| <b>ID #</b> | <b>Initial CM</b> | <b>Reciliation CM</b> | <b>Final CM</b> | <b>nNO (nl/min)</b> |
|-------------|-------------------|-----------------------|-----------------|---------------------|
| 7004        | Abnormal          |                       | Abnormal        | 1.2                 |
| 7027        | Abnormal          |                       | Abnormal        | Not available       |
| 7035        | Not available     |                       | Not available   | 1.6                 |
| 7040        | Normal            |                       | Normal          | 1.6                 |
| 7042        | Abnormal          |                       | Abnormal        | Not available       |
| 7053        | Normal            |                       | Normal          | 1.9                 |
| 7057        | Not available     |                       | Not available   | 5.6                 |
| 7058        | Abnormal          |                       | Abnormal        | 62.8                |
| 7059        | Not available     |                       | Not available   | 118.8               |
| 7102        | Abnormal          |                       | Abnormal        | 87.6                |
| 7113        | Abnormal          | Abnormal              | Abnormal        | 28.1                |
| 7129        | Normal            | Normal                | Normal          | Not available       |
| 7130        | Normal            |                       | Normal          | Not available       |
| 7134        | Abnormal          |                       | Abnormal        | Not available       |
| 7146        | Normal            | Normal                | Normal          | Not available       |
| 7151        | Normal            | Abnormal              | Abnormal        | Not available       |
| 7168        | Normal            | Normal                | Normal          | 10.4                |
| 7190        | Normal            |                       | Normal          | 10.3                |
| 7199        | Normal            |                       | Normal          | 10.3                |
| 7208        | Abnormal          |                       | Abnormal        | 9.4                 |
| 7225        | Normal            | Normal                | Normal          | 18.5                |
| 7242        | Abnormal          |                       | Abnormal        | 16.1                |
| 7253        | Abnormal          |                       | Abnormal        | 11.0                |
| 7266        | Abnormal          | Normal                | Normal          | 17.4                |
| 7281        | Abnormal          |                       | Abnormal        | 9.6                 |
| 7288        | Abnormal          |                       | Abnormal        | 14.6                |
| 7295        | Abnormal          | Abnormal              | Abnormal        | 11.2                |
| 7302        | Normal            |                       | Normal          | 5.8                 |
| 7306        | Abnormal          |                       | Abnormal        | 4.6                 |
| 7319        | Abnormal          |                       | Abnormal        | 16.7                |
| 7336        | Normal            | Normal                | Normal          | 20.0                |
| 7351        | Abnormal          | Normal                | Normal          | 4.6                 |
| 7374        | Normal            | Not yet scored        | Normal          | 13.4                |
| 7376        | Normal            |                       | Normal          | 8.2                 |
| 7379        | Normal            |                       | Normal          | 11.3                |
| 7380        | Normal            |                       | Normal          | 20.8                |
| 7381        | Normal            |                       | Normal          | 12.0                |
| 7386        | Normal            |                       | Normal          | 2.8                 |
| 7389        | Normal            |                       | Normal          | 9.7                 |
| 7397        | Normal            |                       | Normal          | 11.4                |
| 7400        | Normal            | Normal                | Normal          | 9.3                 |
| 7401        | Normal            | Normal                | Normal          | 15.8                |

|             |               |          |               |      |
|-------------|---------------|----------|---------------|------|
| <b>7405</b> | Normal        |          | Normal        | 29.9 |
| <b>7409</b> | Abnormal      | Abnormal | Abnormal      | 10.2 |
| <b>7411</b> | Normal        | Normal   | Normal        | 14.9 |
| <b>7414</b> | Normal        | Normal   | Normal        | 12.1 |
| <b>7415</b> | Normal        | Normal   | Normal        | 18.0 |
| <b>7421</b> | Normal        |          | Normal        | 14.6 |
| <b>7422</b> | Normal        |          | Normal        | 1.8  |
| <b>7425</b> | Normal        |          | Normal        | 16.7 |
| <b>7428</b> | Abnormal      |          | Abnormal      | 19.4 |
| <b>7433</b> | Normal        | Normal   | Normal        | 12.1 |
| <b>7437</b> | Normal        |          | Normal        | 28.7 |
| <b>7438</b> | Normal        | Normal   | Normal        | 12.2 |
| <b>7441</b> | Abnormal      | Abnormal | Abnormal      | 22.5 |
| <b>7444</b> | Normal        | Normal   | Normal        | 18.9 |
| <b>7447</b> | Normal        | Normal   | Normal        | 12.9 |
| <b>7448</b> | Not available |          | Not available | 19.3 |
| <b>7449</b> | Normal        |          | Normal        | 15.1 |
| <b>7450</b> | Normal        | Normal   | Normal        | 15.3 |
| <b>7458</b> | Normal        | Normal   | Normal        | 3.5  |
| <b>7459</b> | Normal        |          | Normal        | 8.0  |
| <b>7466</b> | Normal        |          | Normal        | 20.0 |



**Supplemental Table 4.** Postoperative medication use subcategorized by ciliary motion and nasal nitric oxide

|                            | Events with CM Data<br>(n=91) |                    | Events with nNO Data<br>(n=83) |               |
|----------------------------|-------------------------------|--------------------|--------------------------------|---------------|
|                            | Normal<br>(n=58)              | Abnormal<br>(n=33) | Normal<br>(n=48)               | Low<br>(n=35) |
| Budesonide, n(%)           | 1 (1.7%)                      | 2 (6.1%)           | 0 (0%)                         | 2 (6.0%)      |
| Cisatracurium, n(%)        | 44 (75.9%)                    | 22(66.7%)          | 32 (66.7%)                     | 27 (77.1%)    |
| Epinephrine , n(%)         | 55 (94.8%)                    | 31 (93.9%)         | 45 (93.8%)                     | 33 (94.3%)    |
| Dexamethasone, n(%)        | 20 (34.5%)                    | 10 (30.3%)         | 18 (37.5%)                     | 10 (28.6%)    |
| Heliox, n(%)               | 6 (10.3%)                     | 0 (0%)             | 4 (8.3%)                       | 2 (5.7%)      |
| Hydrocortisone, n(%)       | 19 (32.8%)                    | 3 (9.1%)           | 14 (29.2%)                     | 10 (28.6%)    |
| Inhaled Nitric Oxide, n(%) | 20 (34.5%)                    | 4 (12.1%)          | 13 (27.1%)                     | 10 (28.6%)    |
| Methylprednisolone, n(%)   | 5 (8.6%)                      | 1 (3.0%)           | 3 (6.2%)                       | 3 (8.6%)      |
| N-Acetylcysteine, n(%)     | 2 (3.4%)                      | 4 (12.1%)          | 2 (4.2%)                       | 1 (2.9%)      |
| Nitroprusside, n(%)        | 11 (19.0%)                    | 12 (36.4%)         | 9 (18.8%)                      | 11 (31.4%)    |
| Racemic epinephrine, n(%)  | 8 (13.8%)                     | 1 (3.0%)           | 4 (8.3%)                       | 5 (14.3%)     |
| Sildenafil, n(%)           | 1 (1.7%)                      | 1 (3.0%)           | 2 (4.2%)                       | 0 (0%)        |
| Surfactant, n(%)           | 4 (6.9%)                      | 5 (15.2%)          | 7 (14.6%)                      | 4 (11.4%)     |

Data are median (IQR) or n (%).

**Supplemental Table 5.** Postsurgical outcomes subcategorized by ciliary motion and nasal nitric oxide

|  | Events with CM Data<br>(n=91) |                    | Events with nNO Data<br>(n=83) |                |
|--|-------------------------------|--------------------|--------------------------------|----------------|
|  | Normal<br>(n=58)              | Abnormal<br>(n=33) | Normal<br>(n=48)               | Low<br>(n=35)  |
| CICU Readmission, n (%)                | 7 (12.1%)                     | 3 (9.1%)           | 3 (6.2%)                       | 5 (14.3%)      |
| Chest Tube (days)                      | 6.5 (5-9)                     | 6 (4-8)            | 6 (5-8)                        | 6 (4.5-9.5)    |
| Chest Tube Drainage (mL)               | 626 (347-983)                 | 486 (186-955)      | 606 (339-957)                  | 486 (234-1041) |
| Diaphragm Paralysis, n (%)             | 0 (0%)                        | 2 (6.1%)           | 0 (37.5%)                      | 2 (5.7%)       |
| Fungal Infection, n (%)                | NA <sup>§</sup>               | NA <sup>§</sup>    | 0 (0%)                         | 1 (2.9%)       |
| Intrinsic Airway Disease, n (%)        | 5 (8.8%)                      | 3 (9.1%)           | 4 (8.5%)                       | 4 (11.4%)      |
| Nasogastric Tube <sup>  </sup> , n (%) | 31 (53.4%)                    | 20 (60.6%)         | 27 (56.2%)                     | 21 (60.0%)     |
| Necrotizing Enterocolitis, n (%)       | 4 (6.9%)                      | 3 (9.1%)           | 4 (8.3%)                       | 3 (8.5%)       |
| Pleural Effusion, n (%)                | 15 (25.9%)                    | 6 (18.2%)          | 10 (20.8%)                     | 10 (28.6%)     |
| Pneumonia, n (%)                       | 0 (0%)                        | 2 (6.1%)           | 0 (0%)                         | 0 (0%)         |
| Pneumothorax, n (%)                    | 1 (1.7%)                      | 3 (9.1%)           | 2 (4.2%)                       | 0 (0%)         |
| Tracheitis, n (%)                      | 1 (1.7%)                      | 2 (6.1%)           | 0 (4.2%)                       | 2 (5.7%)       |
| Tracheostomy, n (%)                    | 1 (1.7%)                      | 0 (0%)             | 0 (0%)                         | 1 (4.5%)       |

Data are median (IQR) or n (%).

<sup>§</sup> NA = not available as the single fungal infection occurred in a patient who did not have ciliary motion sample

<sup>||</sup> Need for nasogastric tube at time of hospital discharge in order to meet nutritional requirement

**Supplemental Table 6.** Detailed postoperative infection data for study participants with infection

| <b>ID #</b> | <b>Surgical Encounter</b> | <b>Infection Type</b> | <b>Organism</b>                                   | <b>Site</b> |
|-------------|---------------------------|-----------------------|---|-------------|
| 7004        | 1                         | Bacterial             | <i>Enterococcus faecalis</i>                      | Blood       |
| 7004        | 2                         | Bacterial             | <i>Coagulase-negative staphylococcus</i>          | Blood       |
| 7027        | 1                         | Bacterial             | <i>Vancomycin-resistant enterococcus</i>          | Urine       |
| 7035        | 1                         | Fungal                | <i>Candida albicans</i>                           | Blood       |
| 7040        | 1                         | Bacterial             | <i>Pseudomonas aeruginosa</i>                     | Urine       |
| 7042        | 1                         | Bacterial             | <i>Stenotrophomonas maltophilia</i>               | CSF         |
| 7058        | 1                         | Bacterial             | <i>Enterococcus faecalis</i>                      | Mediastinum |
| 7102        | 1                         | Bacterial             | Culture negative                                  | Mediastinum |
| 7113        | 1                         | Bacterial             | <i>Moraxella catarrhalis</i>                      | Trachea     |
| 7113        | 3                         | Viral                 | <i>Rhinovirus/Enterovirus</i>                     | Nares       |
| 7134        | 1                         | Bacterial             | <i>Streptococcus pneumoniae</i>                   | Blood       |
| 7134        | 1                         | Bacterial             | <i>Proteus mirabilis</i>                          | Urine       |
| 7134        | 1                         | Viral                 | <i>Rhinovirus/Enterovirus</i>                     | Trachea     |
| 7134        | 2                         | Bacterial             | <i>Coagulase-negative staphylococcus</i>          | Mediastinum |
| 7134        | 2                         | Viral                 | <i>Respiratory syncytial virus</i>                | Nares       |
| 7168        | 1                         | Bacterial             | <i>Coagulase-negative staphylococcus</i>          | Blood       |
| 7168        | 3                         | Bacterial             | <i>Coagulase-negative staphylococcus</i>          | Mediastinum |
| 7199        | 1                         | Bacterial             | Culture negative                                  | Blood       |
| 7242        | 1                         | Bacterial             | Culture negative                                  | Abdomen     |
| 7302        | 1                         | Bacterial             | Culture negative                                  | Mediastinum |
| 7319        | 1                         | Bacterial             | <i>Coagulase-negative staphylococcus</i>          | Blood       |
| 7351        | 2                         | Bacterial             | <i>Klebsiella oxytoca</i>                         | Urine       |
| 7376        | 2                         | Bacterial             | <i>Pseudomonas aeruginosa/ Klebsiella oxytoca</i> | Trachea     |
| 7386        | 1                         | Bacterial             | Culture negative                                  | Mediastinum |
| 7389        | 1                         | Bacterial             | Culture negative                                  | Abdomen     |
| 7401        | 1                         | Bacterial             | Culture negative                                  | Mediastinum |
| 7430        | 1                         | Viral                 | <i>Rhinovirus</i>                                 | Nares       |
| 7438        | 2                         | Bacterial             | <i>Enterococcus faecalis</i>                      | Blood       |
| 7449        | 1                         | Bacterial             | Culture negative                                  | Abdomen     |
| 7450        | 1                         | Bacterial             | Culture negative                                  | Mediastinum |
| 7466        | 1                         | Bacterial             | <i>Coagulase-negative staphylococcus</i>          | Blood       |

### **Supplemental Reference**

1. Yatsenko SA, Davis S, Hendrix NW, Surti U, Emery S, Canavan T, Speer P, Hill L, Clemens M and Rajkovic A. Application of chromosomal microarray in the evaluation of abnormal prenatal findings. *Clinical genetics*. 2013;84:47-54.