Urine as a specimen to diagnose infections in twenty-first century: focus on analytical accuracy

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Urine as a clinical specimen to diagnose infections has been used since ancient times. Many rapid technologies to assist diagnosis of infections are currently in use. Alongside traditional enzyme immunoassays (EIA), new technologies have emerged. Molecular analysis of transrenal DNA to diagnose infections is also a rapidly growing field. The majority of EIAs utilize the detection of excreted sugar compounds of the outer microbial cell-wall shed into the bloodstream and excreted into the urine. This mini-review focuses on current knowledge on rapid urinary antigen detection tests to diagnose most common infections, and highlights their diagnostic utility. The past and the future of urinalysis are also briefly discussed. The analysis of the literature shows that some methods are not quantitative, and analytical sensitivity may remain suboptimal. In addition, the performance criteria and technical documentation of some commercial tests are insufficient. Clinical microbiologists and physicians should be alert to assay limitations.

Keywords: urinalysis, urine, parasites, bacteria, viruses, fungi, immunochromatographic methods, ELISA

URINE AS A SPECIMEN

Milleniums ago urine as a diagnostic specimen attracted the attention of early physicians. The history of uroscopy and urinalysis starting from the time of Pharaonic Egypt has been reviewed (White, 1991; Eknoyan, 2007). In Egypt and Aztec cultures urine was used to disinfect wounds and was rubbed into the skin to treat cuts and burns (Aztecs at Mixicoloro by Bernard Ortiz de Montellano, Wayne State University, USA; Available at: http://www.mexicolore.co.uk/index.php?one=azt&two=hea). In medical papyri (1550 BCE) of ancient Egypt hematuria was described using hieroglyphic symbols. This condition was most probably caused by the worm *Schistosoma haematobium* (Papyrus, 1875) that plagued people living along the Nile Valley (Ruffer, 1910).

A Greek philosopher, naturalist, and the Roman emperor's physician, Claudius Galenos (129–200 CE) used the humoral philosophy of ancient Greece which was based on the concept of Hippocrates (460–370 BCE). According to Hellenistic teaching the body is at equilibrium or homeostasis when four juices are in balance. Urine was one of the four pillars of the galenic humoral pathology. Centuries later this teaching was reintroduced in Europe during the Renaissance.

Urine is an ideal clinical specimen for diagnostics because it is excreted in large quantities and collection does not require invasive methods. When conventional culture remains either negative or unreliable, diagnosis of systemic infectious disease based on the demonstration of bacterial antigens directly from urine has many potential advantages. If the kidneys are intact, the excreted urinary antigens have a lower MW than of albumin (<67 kDa) or for transrenal DNA usually do not exceed 100 bp (Green et al., 2009).

This mini-review describes several methods for some important infections, with the aim to illustrate their analytical utility. Regarding the extent of the contemporary literature it cannot be comprehensive.

URINE RAINBOW

Inspection of the color of urine is still useful today (Foot and Fraser, 2006). Unusual color comprising practically the whole spectrum of the rainbow is associated with various metabolic or infectious diseases. Below are illustrated only a few examples where the color of the urine is almost pathognomonic for a specific infection.

RED COLOR, OR HEMATURIA

Hematuria is a condition of the presence of fresh blood in urine. This symptom is found in a great variety of non-infectious disorders but may also occur in relation to common infections, e.g., urinary tract infection (UTI). It may follow the prodromal period (Katayama syndrome) after infection with *Schistosoma haematobium* (CDC[1], WHO: schistosomiasis[2]). Hematuria may also be a symptom in viral zoonotic infections that may lead to hemorrhagic fever with renal syndrome (HFRS). HFRS are caused by, e.g., Hantaan, Dobrava, Seoul viruses, or other arboviruses, e.g., Yellow Fever Disease, Rift Valley Fever, or tick-borne Crimea-Congo Hemorrhagic Fever, or Dengue virus that may cause Hemorrhagic Fever (DHF; WHO: Dengue[3]).

BROWN COLOR, OR BLACK WATER FEVER AND OTHER INFECTIONS

Black water fever is a urological manifestation of malaria caused by *Plasmodium falciparum*. The color of urine turns brownish due

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[1]www.cdc.gov/parasites/schistosomiasis/gen_info/faqs.html
[2]http://www.who.int/mediacentre/factsheets/fs115/en/index.html
[3]http://www.who.int/mediacentre/factsheets/fs117/en/

to profuse hemoglobinuria. The brownish color of urine could be observed also in cholestasis with jaundice that may occur, e.g., in hepatitis A virus infection or in leptospirosis, a zoonotic disease, caused by the spirochete *Leptospira interrogans*.

MILKY COLOR, OR CHYLURIA

Chyluria, or "milky urine", is a symptom of the disturbance of lymphatic flow that may be caused by filariasis. A tapeworm, *Wuchereria bancrofti*, accounts for 90% of human filariasis and is widespread across tropic and subtropic areas (Kehinde et al., 2008).

INFLAMMATORY MARKERS FROM URINE

A non-specific immune marker, neopterin, produced by human macrophages has been detected from urine as well as blood. This marker was found elevated in several inflammatory diseases and in AIDS (Fuchs et al., 1989). Recently, pro-inflammatory chemokines and cytokines have been studied in urine from patients with pulmonary tuberculosis (TB). Only IP-10 was excreted in the urine in measurable quantities exceeding those of patients without TB (Cannas et al., 2010).

TESTS FOR HISTOPLASMOSIS

Progressive disseminated histoplasmosis (PDH) is common in persons with AIDS, and other immunosuppressed patients (Kauffman, 2008). Antigen detection in plasma or urine is a significant adjunct to clinical and other diagnostic methods. The most frequently reported test is the enzyme immunoassay (EIA) developed by MiraVista Diagnostics (Indianapolis, IN, USA) which has been recently refined (Wheat et al., 2007). This method detects circulating polysaccharides of *Histoplasma capsulatum* and provides quantification of antigen concentration. A quantitative assay that can measure the polysaccharide down to 0.6 ng/ml (range 0.6–39) was recently reported (Connolly et al., 2007). The manufacturer of another commercially method, IMMY ALPHA (Immunol-Mycologics, Inc., Norman, Oklahoma, USA), claims similar performance although some authors reported controversy (McKinsey et al., 2009).

Antigenuria is a sensitive laboratory biomarker of *Histoplasma* clearance and was used to monitor the efficacy of antimycotic treatment (Hage et al., 2011a). On the other hand, positive urine antigen levels may persist at low levels for months in patients without clinical illness (Kauffman, 2008). In a large evaluation in cases with acute pulmonary histoplasmosis (APH) the sensitivity of both urine and blood detection methods was equal, being positive in 64.6% (antigenuria) and 68.6% (antigenemia; Swartzentruber et al., 2009). Combined, these tests reached a sensitivity of 82.8% but, combined with the antibody detection method, the sensitivity was 93.3% (Swartzentruber et al., 2009). In culture positive patients with PDH the sensitivity of the ELISA urine method was higher but still suboptimal, 81%, but the specificity in healthy persons and non-histoplasmosis disease reached 95% (Scheel et al., 2009). In a multicenter evaluation the sensitivity of antigen test was proportional to disease severity, 91.8% in PDH and only 30.4% in subacute histoplasmosis (Hage et al., 2011b). It was presumed that antigenuria should be greater and, thus, more readily detectable in patients with a high fungal burden such as in

AIDS patients with PDH (sensitivity ∼90%) than in other patients (e.g., pulmonary forms, sensitivity ∼75%; Kauffman, 2008). This observation corroborates the findings from TB studies when urinary lipoarabinomannan (LAM) was detected more readily from AIDS patients than from others. Similarly to pneumococcal antigen detection methods, ultrafiltration of urine was suggested to improve sensitivity (Srinivasan et al., 2009). As expected, due to structural similarities of polysaccharides from other dimorphic fungi (*Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, and *Penicillium marneffei*) some interference from cross-reactivity has been documented (Kauffman, 2008).

OTHER URINARY TESTS FOR DISSEMINATED MYCOSES

In invasive aspergillosis chest x-ray, computer tomography, culture, and microscopy may not always lead to diagnosis. The commercial Platelia™*Aspergillus* ELISA detects galactomannan from *Aspergillus* spp. The method was tested with 105 samples from hematological patients using sera, urine, or bronchoalveolar lavage. Serum and urine analyses were positive in 14 and 5% of samples, respectively. Ten-fold concentration of urine increased the number of positive samples to 8%. In six patients with confirmed invasive aspergillosis the antigen was detected in serum, but only in two was the urine test positive, suggesting serum is a preferred sample for screening (Salonen et al., 2000).

A urine test to detect *B. dermatitidis* antigen (Durkin et al., 2004) has a sensitivity of 92% (in pulmonary and in disseminated forms and good specificity in healthy volunteers but interference from cross-reactivity occurred in 96.3% of patients with histoplasmosis, 100% in paracoccidioidomycosis, 70% in penicilliosis, and very low cross-reactivity in patients with cryptococcosis and aspergillosis (Durkin et al., 2004).

Another capture-based EIA detects antigens from *C. immitis* (Durkin et al., 2009). This test was applied both to serum and urine. The authors claimed that they were able to measure galactomannan from urine samples at concentrations down to 0.07 ng/ml yielding clinical sensitivity of 50%.

In summary, results of tests for fungal antigenuria are promising. However, the clinical relevance of some methods is difficult to reproduce by outside laboratories because they are proprietary to the companies (Scheel et al., 2009).

STREPTOCOCCUS PNEUMONIAE POLYSACCHARIDES IN URINE

Despite availability of pneumococcal vaccines, *Streptococcus pneumoniae* is the number one causative agent of community acquired pneumonia (CAP), sepsis, and meningitis. The diagnostic utility of excreted soluble urine antigen in patients with pneumonia was first described in 1917 (Dochez and Avery, 1917). Current diagnostic aids have been recently reviewed (Klugman et al., 2008). According to the authors the sensitivity of all methods to identify *S. pneumoniae* in CAP remains inadequate. Bacterial isolation from patients with pneumococcal CAP was successful from less than 20% (Klugman et al., 2008) to 37.5% (Marcos et al., 2003). Urinary diagnostics can be a useful adjunct to culture. A positive result is highly indicative of *S. pneumoniae* as the causative agent in adults but less so in infants due to microbial colonization in their nasopharyngeal cavity (Klugman et al., 2008). The urinary test is based on the detection of polysaccharide C common in all clinically

significant serogroups. For example, immunochromatographic membrane assay (ICT) Binax® NOW *S. pneumonia* Urinary Antigen Test (ME, USA) using blood culture as the gold standard for positives displayed a sensitivity of 86–90% and specificity 71–94%, the latter being dependent on the study cohort. In a large study (Marcos et al., 2003) the sensitivity reached 100% in patients with definitive pneumococcal pneumonias and 69.2% in patients with probable pneumonia, whereas in patients without pneumococcal pneumonia a false positive rate of 18% was observed. These authors further advocated the use of concentrated urine; however, they did not clearly address the issue of specificity. The Binax® NOW provides qualitative antigen identification and analytical sensitivity is expressed in terms of colony forming units/ml which is not relevant to the quantities of excreted antigen in terms of concentration. Concerning specificity, the manufacturer reported that urine from patients with UTI displayed variable interference (9%) resulting in invalid result interpretations. Interestingly, urine collected from persons without signs of UTI may also occasionally test false positive although culture negative. The reason for this interference is presently obscure. One possibility is that the cross-reactivity is produced by thus far uncharacterized host inflammatory reaction compounds or alimentary components, rather than bacterial constituents.

The issue of urine concentration remains highly controversial. The manufacturer recommends the use of non-concentrated urine, but some researchers (Domínguez et al., 2001; Marcos et al., 2003) claimed that a 25-fold urine concentration may provide increased sensitivity. In a large prospective evaluation (Marcos et al., 2003) concentrated urine resulted in a 1.4-fold increase in positives detected (from 38 to 53%). However, large prospective evaluation (Murdoch et al., 2001) using concentrated urine increased positive detection only marginally (from 29 to 30%) in CAP patients.

Based on ultrafiltration experiments the size of the C polysaccharide of approximately 20–30 kDa was suggested (Murdoch et al., 2001). Pioneering work performed at The Rockefeller Institute (NY, USA) in the early 1960s (Gotschlich and Liu, 1967) characterized the polysaccharide C extracted from bacterial culture. Later, (Sørensen et al., 1990; Karlsson et al., 1999) the polysaccharide C was studied in details. Still unresolved is the size and the chemical composition of the antigen that is excreted into the urine.

LEGIONELLA PNEUMOPHILA POLYSACCHARIDES IN URINE

Suspicion of nosocomial or community acquired legionellosis warrants immediate diagnosis and differentiation from other pathogens because the infection requires antibiotic treatment different from that used in pneumococcal infection.

There are several commercial urinary antigen detection tests on the market, the majority being developed for the clinically most important *Legionella pneumophila* serogroup 1. For example, Binax® Now *Legionella* Urinary Antigen Test (Inverness Medical) is a ICT with a low interference (5%) in cohorts of bacterial pneumonia, sepsis, and UTI. When the three ICT antigen detection tests were compared using 39 unfrozen and non-concentrated urine samples from patients with Legionnaire's disease, the sensitivities were 74.3, 61.5, and 41% for the Binax® Now, the SAS™*Legionella*

Test (SA Scientific Inc.) and the Uni-Gold™*Legionella* Urinary Antigen Plus (Trinity Biotech; Muñoz et al., 2009). However, recent comparison of the Binax® NOW ICT to two EIA methods resulted in superior EIA performance. This retrospective study was performed on 178 frozen urine samples. The authors found that with non-concentrated urine samples the sensitivity levels of 71.3% for the Bartels *Legionella* Urinary Antigen (Trinity Biotech); 65.1% for Biotest *Legionella* Urine Antigen EIA (now marketed by BioRad) and 37% for Binax® NOW. After concentration, however, significant differences in sensitivity no longer existed (Guerrero et al., 2004). The specificity issues of concentrated vs. non-concentrated samples are presently unrecognized.

A systematic review and meta-analysis assessed the performance of EIA-based and ICT urine antigen detection methods (Shimada et al., 2009). The authors concluded (Shimada et al., 2009) that higher quality studies had lower sensitivity, and a publication bias was evident. In their summary the pooled sensitivity of urinary methods to detect infection with *L. pneumophila*, serotype 1 was 74% (95% CI, 68–81%), and the specificity was 99.1% (95% CI, 98.4–99.7%).

As a matter of fact, the origin of the test antigen is not reported in commercial protocols; however, from its strict serogroup specificity one may assume that it is lipopolysaccharide (LPS), a heat-stable antigen present in all Gram-negative bacteria. Chemical analysis of *Legionella* LPS was performed decades ago (Conlan and Ashworth, 1986), but the size and the chemical composition of the excreted polysaccharide are not yet known.

LAM FROM URINE TO ASSIST DIAGNOSIS OF TUBERCULOSIS

Tuberculosis is a global health emergency of enormous proportions. The major obstacles to TB control is the lack of fast, affordable, and accurate diagnostic techniques both for pulmonary and extrapulmonary TB in adults and children (Peter et al., 2010; Yasinskaya et al., 2011). One approach is to detect the presence of urinary microbial metabolites, e.g., LAM.

Lipoarabinomannan is a structural heat-stable cell-wall glycolipid of *Mycobacterium tuberculosis*. It constitutes up to 15% of the total bacterial weight. LAM is a virulence factor and is released from metabolically active or degrading *M. tuberculosis* in TB infection. The size of this polymeric structure is variable, being on the average 17.5 kDa (range 6–34; Chatterjee, 1997; Brennan, 2003; Nigou et al., 2003). The first detection of urinary LAM was described a decade ago (Hamasur et al., 2001). An earlier commercial method (Chemogen Inc., Portland, ME, USA), is presently marketed as the Clearview™TB ELISA (Inverness Medical Innovations). A meta-analysis and systemic review of the diagnosis of TB using this reagent reported estimates of sensitivity and specificity in microbiologically confirmed cases (seven studies) of 13–93 and 87–99%, respectively (Minion et al., 2011). In five studies with results stratified by HIV status, the sensitivity was 3–53% but in HIV +ve subgroups it was systematically higher and the highest sensitivity was observed in advanced immunosuppression. In a large prospective evaluation including 141 patients with definitive TB and 172 patients with no proven TB, urine LAM test was positive in only 17/141 and 1/172, respectively (Dheda et al., 2010). In this study the sensitivity in HIV +ve patients was 21% and in

those whose CD4+ cell counts were below 200 cell/mm^3 the sensitivity reached 37%; the specificity being 95–100% (Dheda et al., 2010). Reported data have shown that better diagnostic utility is associated with advanced stages of HIV infection and low CD4+ cell count (Dheda et al., 2010). This observation is explained by an assumption that in TB with AIDS as co-morbidity, bacterial burden is higher compared to cases without AIDS. Amazingly, extensive clinical evaluations (Minion et al., 2011) have been performed using the Clearview™TB ELISA without confirmation that LAM is, in fact, excreted in urine of TB patients. No evidence is apparent also for LAM as the analyte detected by the polyclonal antibodies is the LAM indeed. As in the *S. pneumoniae* urine detection method cross-reactivity with possibly structurally non-related substances is observed. This finding may further lead to speculation that mechanisms of assay interference could be common for assays of this type where polyclonal antibodies are used.

URINE AS A SAMPLE TO STUDY VIRAL INFECTIONS

Human polyomaviruses (HPyVs) are widespread and unique to humans. High quantities of viral particles can be shed in urine from a healthy individual. In nephropathy, e.g., after kidney transplantation, in profound immunosuppression or in AIDS, in hemorrhagic cystitis (BKV), or in symptoms of central nervous system compatible with progressive multifocal leucomyelopathy (JCV), these viruses may no longer be "innocent bystanders" (Kumar, 2010). NAATs for both urine and blood have been developed to detect, subtype, and estimate viral loads.

Congenital cytomegalovirus (CMV) infection, a leading cause of sensorineural hearing loss and neurological impairment (Cannon et al., 2011), can be robustly detected directly from urine. In primary infections CMV is excreted and can be cultured from urine for several weeks.

Urine has been utilized as sample for the early diagnosis of Dengue virus infection. In some situations urine NAATs provided greater sensitivity than does analysis of plasma (Poloni et al., 2010). Virus-specific IgG or IgA-class antibodies as serological markers of this disease have been also detected in urine (Vazquez et al., 2007).

URINE AS A SAMPLE TO DIAGNOSE SEXUALLY TRANSMITTED DISEASES

Urine is extremely useful to diagnose infections of the reproductive tract. The recent systematic review (Cook et al., 2005) on non-invasive testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* showed that both assays reached specificities of 97% for both infections and in both sexes. However, for chlamydial infection the pooled sensitivities were: 83.3, 92.5, and 79.9% for PCR, transcription-mediated amplification and strand displacement amplification assays in women and 84, 87.7, and 93.1% in men, respectively. For the gonococcal infection the respective sensitivities in women were 55.6, 91.3, and 84.9%. Therefore, PCR for gonococcal infection in women performed from urine was not recommended (Cook et al., 2005).

The newly developed triple assay for the simultaneous detection of *C. trachomatis, N. gonorrhoeae,* and *Mycoplasma genitalium* (Dx CT/NG/MG assay BioRad, USA) may provide more evidence about the role of *M. genitalium* in sexually transmitted diseases (STDs). This smallest human bacterium has been earlier associated with non-gonococcal urethritis in men but now also in low-risk women. *M. genitalium* is considered a cause of urethritis, mucopurulent cervicitis, and upper genital tract infections like pelvic inflammatory disease and even infertility (McGowin and Anderson-Smits, 2011; Weinstein and Stiles, 2011).

EMERGING TECHNOLOGIES AND PATHOGENS

Flow cytometry-based analysis (FACS) that quantifies both bacteria and white blood cells in the urine has emerged as a promising diagnostic tool (Jolkkonen et al., 2010). Gas chromatography/mass spectrometry to detect volatile organic components in human urine has been recently described although not yet implemented in clinical practice (Banday et al., 2011). Detection of microbial transrenal DNA is an attractive option to diagnose infections (Green et al., 2009). The authors, however, cited concerns about lack of standardization of methodology both for pre- and analytical steps, storage, and transportation of the sample. A nested PCR from urine and saliva to detected trace amounts of *Plasmodium* DNA has been described (Putaporntip et al., 2011). The sensitivity of the urine method was two to fourfold less than that shown for saliva, but the combination of both biological fluids displayed a sensitivity superior to that of microscopy. Moreover, double malarial infections could be easily detected.

As new detection technologies emerge, knowledge about new pathogens continues to increase. For example, *Actinobaculum schaalii* may cause undiagnosed cystitis predominantly in the elderly and in those persons in whom the primary focus appears to be anomalies in the genitourinary tract (e.g., benign prostatic hyperplasia). Most recently it has been associated with bloodstream infections (Beguelin et al., 2011). Urine FACS may prove useful to identify less known or unknown pathogens which can be suspected on the basis of discrepancy between conventional culture and a positive fluorescence signal denoting "presence" of bacteria.

CONCLUSION

In the future urine, because of ease of collection, processing, and storage, may be extensively used for targeted multiplexed analyses. Current state-of-the-art, however, indicates that some detection methods still require further understanding of molecular forms and structures detected, as well as the excretion kinetics of these polymeric compounds.

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