Measuring Salivary Testosterone on a National Population Survey

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On behalf of the Natsal team
Overview

• British National Survey of Sexual Attitudes and Lifestyles (Natsal-3)
• What is testosterone and how can it measured?
• Laboratory challenges
• Fieldwork challenges
• The Natsal-3 approach to measuring testosterone
The National Survey of Sexual Attitudes and Lifestyles (Natsal)

- Probability sample survey of British Population aged 16-74
- 1 adult per household (random selection)
- 15,162 interviews carried out 2010-’12
- Response rate: 57.7%
- CAPI / CASI Interview about sexual experiences, behaviour, attitudes
- Token of appreciation: £15 voucher
The Natsal Team

- Epidemiologists
- Social scientists
- Public Health scientists
- Clinicians
- Clinical scientists
- Survey experts
- Qualitative researchers
- Endocrinologists
Funding and ethical approval

- Grants from Wellcome Trust and Medical Research Council
- Includes funding from Department of Health and Economic and Social Research Council
- Ethical approval via National Research Ethics Service
Natsal-3 Biomarkers

Saliva samples $\rightarrow$ Testosterone

Urine samples $\rightarrow$ Sexually Transmitted Infections
What is Testosterone?
What is Testosterone?

- Sex steroid hormone
- Produced by men and **women**
- Variety of roles throughout life course

Smaller quantities
Why measure Testosterone on Natsal?

1. Examine relationship between T levels and sexual function and behaviour in men and women
2. Document prevalence of lack of testosterone in men (hypogonadism)
3. Establish the normative range of free T across the life span in men and women
Natsal-3 Testosterone aims

• Measure Testosterone in 4,400 men and women aged 18-74
• Link Testosterone result to survey data
Measuring Testosterone

Less invasive
Can be interviewer- / self-administered (less costly)
Higher response rate?
Can Testosterone be measured accurately in Saliva?
Circulating Testosterone in blood

Free T: unbound fraction can be measured directly or calculated from total T and binding proteins.

Salivary T: reflects Free T in the circulation
Laboratory challenges

• Validation of saliva samples needed
  – Natsal Validation study showed good correlation between measured salivary free-T and calculated serum free-T

![Graph showing correlation between SalT and Serum free-T](image)

- $r = 0.71$
- $y = 1.1x + 0.15$
Laboratory challenges

• Validation of saliva samples needed
  – *Natsal Validation study showed good correlation between measured salivary free-T and calculated serum free-T*

• Low levels in saliva = harder to detect (need more sensitive test)

• Specificity also important
  – *Mass Spectrometry (LC MS/MS) sensitive and specific*
Letters to the Editor

A Liquid Chromatography–
Tandem Mass Spectrometry
Method for Salivary
Testosterone with Adult Male
Reference Interval
Determination

To the Editor:

Our laboratory has extensive experience in developing liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods for routine use in the clinical setting, and did not brush their teeth 30 min before sampling. Samples were collected by drooling down a plastic straw into a collection vial; the saliva sample was then frozen at −80 °C. Samples were thawed, mixed, and centrifuged; the clear supernatant was then used for analysis.

Sample preparation involved a liquid–liquid extraction requiring 200 μL sample with D₅-testosterone internal standard and methyl-tert-butyl ether, which was added. The recoveries of testosterone were within 15% of the target values. The mean recoveries from saliva samples (n = 6) at 3 concentrations were 95.6%, 100.3%, and 95.8%, respectively.

Sample-stability studies showed no appreciable loss in testosterone, a finding supporting the feasibility of patients producing saliva samples at home and sending them to appointed laboratories. A comparison of our LC-MS/MS assay with an established Sal-T RIA
Operational challenges

Non-clinical setting

• Sample stability
Glasgow Royal Infirmary
Operational challenges

Non-clinical setting

• Sample stability
• Need for an ‘uncontaminated’ sample
• Testosterone levels vary throughout the day (highest in morning)

Balancing need for useable samples with respondent burden / response rates
Overcoming the challenges (1)

Sample stability

– Validation study showed stability over 5 days
– Samples needed to be POSTED SAME DAY
– Avoid public holidays
– Prepared and frozen on receipt by lab, then batch tested
Overcoming the challenges (2)

Diurnal variation (time of day)

Need for uncontaminated samples

- *Take samples first thing in morning (<10am)*
- *Restrict to ESSENTIAL requirements (food/drink and brushing teeth)*
Natsal-3 Saliva study

• Introduced at end of interview
• Self-collection packs
• Simple instructions
• Interviewer training
• Interviewer follow-up phone call
Testing protocols

Pilot 1 (no reminder call)
  – 48% response rate

Pilot 2 (reminder phone call)
  – 61% response rate
Natsal-3 Saliva study

- Introduced at end of interview
- Self-collection packs
- Simple instructions
- Interviewer training
- Interviewer follow-up phone call
- Token of appreciation: £5 voucher once sample received by lab
- Results not returned
Response rates

• Aim: 4,400 useable samples
• Agreement rate (at interview):
  6516/9171 = 71%
• Actual response rate:
  4619/9171 = 50%
• Useable samples*: approx 4470 = ~49%

Is 50% an acceptable response rate?

*preliminary figures
Response rates (2)

• Gender differences?

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>Agreement</td>
<td>71.6%</td>
<td>70.9%</td>
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<tr>
<td>Completion*</td>
<td>51.1%</td>
<td>49.9%</td>
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* % of all invited
Response rates (3)

• Age differences?

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<tr>
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<th>18-24</th>
<th>25-34</th>
<th>35-44</th>
<th>45-54</th>
<th>55-64</th>
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<tbody>
<tr>
<td>Agreement</td>
<td>74.5%</td>
<td>71.5%</td>
<td>67.5%</td>
<td>72.6%</td>
<td>71.8%</td>
<td>69.8%</td>
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<tr>
<td>Completion*</td>
<td>39.2%</td>
<td>42.8%</td>
<td>47.1%</td>
<td>52.6%</td>
<td>59.5%</td>
<td>62.1%</td>
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</table>

* % of all invited
Summary

• Possible, in spite of considerable challenges
• Yielded unique and valuable data
• Trade-off between sample quality and response rates
• Non-response weighting will be very important
• Let the analysis begin!
Questions?
Natsal-3 Biomarkers

Saliva samples → Testosterone

Urine samples → Sexually Transmitted Infections
Testing for STIs in population-based sexual health surveys without returning results: development of an ethical and pragmatic approach

Soazig Clifton, Sven Sjodin (NatCen Social Research)
Nigel Field, Anne Johnson, Catherine Mercer, Pam Sonnenberg, Clare Tanton (University College London)
Simon Beddows, Catherine Ison, Kate Soldan (Public Health England)
On behalf of the Natsal team
Overview

• Natsal STI testing
  – Why
  – How
• Ethical considerations in the non-return of results
• Agreed approach
• Summary of recommendations
• Discussion
Natsal STI testing: aims

To measure the population prevalence of 5 key STIs in urine in a probability sample of men & women

- HIV antibody
- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*
- Type-specific human papillomavirus (HPV)
- *Mycoplasma genitalium*
Diagnoses of new STIs at sexual health clinics by gender, England: 2002-2011

Source: Public Health England
Natsal provides unique data

- Population-based data
- Includes those not in contact with health services
- Link to behavioural and service use data
**Natsal-2 STI testing**

- Urine samples tested for *Chlamydia trachomatis*
- Used to inform National Chlamydia Screening Programme (England)

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**Sexual behaviour in Britain: reported sexually transmitted infections and prevalent genital *Chlamydia trachomatis* infection**

*Kevin A Fenton, Christos Korovessis, Anne M Johnson, Angela McCadden, Sally McManus, Kaye Wellings, Catherine H Mercer, Caroline Carder, Andrew J Copas, Kiran Nanchahal, Wendy Macdowall, Geoff Ridgway, Julia Field, Bob Erens*

**Summary**

**Background** Studies of the epidemiology of sexually transmitted infections (STI) are largely based on surveillance data. As part of a national survey of sexual attitudes and lifestyles (Natsal 2000) in Britain, we estimated the frequency of self-reported STIs, and the prevalence of urinary *Chlamydia trachomatis* infection.

**Introduction**

The epidemiology of sexually transmitted infections (STIs) in populations results from the interaction between biological characteristics of the relevant organisms, the behaviours that transmit them, and the effectiveness of prevention and control interventions.1 In Britain, our understanding of STI epidemiology is largely based on surveillance data obtained from a national network of Genito-urinary Medicine (GUM) clinics. Data from...
Natsal-3 STI testing: methods

- 4,550 samples tested
- Urine sample introduced at end of interview
- Subsample of sexually experienced men and women aged 16-44
- Sample taken while interviewer present
- Used novel firstburst device
- Posted by interviewer
Natsal-3 STI testing: methods

- 4,550 samples tested
- Urine sample introduced at end of interview
- Subsample of sexually experienced men and women aged 16-44
- Sample taken while interviewer present
- Used novel firstburst device
- Posted by interviewer
- Token of appreciation: £5 voucher
- Results not returned
Deciding whether to give participants their test results
Risks of not returning results

• Clinical implications
• Missed opportunity to reduce harm for participants with treatable infections
• Missed opportunity to prevent onward transmission
• Conflict with autonomy over samples
Concerns about returning results

• Accuracy of tests (urine sample - not diagnostic standard)
• Conditions of testing (non-clinical setting; timeliness of testing)
• Wide availability of free testing on National Health Service
• Testing for infections with unknown clinical implications
Concerns about returning results

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# Infection specific issues

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</tr>
<tr>
<td>HIV</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>HPV</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Mycoplasma genitalium</td>
<td>NA</td>
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**Infection specific issues**

Chlamydia, Gonorrhoea, HIV, HPV, Mycoplasma genitalium.
## Infection specific issues

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</tr>
<tr>
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<tr>
<td><em>genitalium</em></td>
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Summary

- Tests accurate at population level
- Possible for small number of false positive / false negative results

Undue reassurance

Undue concern
Summary

• Tests accurate at population level
• Possible for small number of false positive / false negative results
• Diagnostic tests freely available
• Consistent approach across all infections important to avoid misunderstanding
Other considerations

• Problems contacting all participants

11% positive cases non-contactable

• Cost considerations
Other considerations

• Problems contacting all participants

11% positive cases non-contactable

• Cost considerations
Natsal-3 approach

- Voluntary anonymised testing
- Results not returned
Natsal-3 approach

• Voluntary anonymised testing
• Results not returned
• Specific informed written consent
1) I consent to give a urine specimen to the Natsal 2012 survey. This sample will be used to test for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), *Mycoplasma genitalium* (MG), Human Papillomavirus (HPV), and Human Immunodeficiency Virus (HIV) antibody.

The interviewer has explained to me how and why this urine sample is being taken, and I have had a chance to discuss this with him/her. I have also received an information leaflet explaining these matters.

I understand that I will not be given any result performed on my sample. I understand that the result to my name and address, and will not be traced back to me.

**What happens to the results of these tests?**

The test results will be linked to the answers given in the study, but your name, address and other identifying information will be deleted before we see the test results so there is no way to trace the result back to you. We are unable to give you the results of the test because steps we are taking above make the sample anonymous. The data from the tests are strictly confidential and the information will be used for statistical and research purposes only and dealt with according to the principles of the 1998 Data Protection Act.

**Why will my test results not be returned?**

We will not be able to give you any test results because: (1) While the tests are good enough to give information at a population level about these infections, they are not as accurate as the ones used by the NHS for diagnosis and treatment; (2) We are testing for some infections that do not need treatment (3) The results will be made anonymous and will never be linked to your name and address.
Natsal-3 approach

• Voluntary anonymised testing
• Results not returned
• Specific informed written consent
• Information provided on access to STI advice, testing and treatment services
What should I do if I am worried about having a sexually transmitted infection (STI)?

If you have concerns that you may have been infected with an STI, we suggest you seek immediate professional advice. Free testing for sexually transmitted infections, as well as free confidential advice about sexual health, family planning and contraception, can be obtained from your local STI or Genitourinary Medicine (GUM) clinic, your family GP, family planning clinics, as well as some pharmacies and youth centres. The following website will allow you to find your nearest sexual health service: www.nhs.uk/livewell/sexualhealth.

The interviewer will also give you a helpline leaflet which contains some more information about these organisations and their telephone numbers; you can also visit their websites for further information.

The Department of Health (DH) recommends sexual health check-ups for sexually active people every time they change partner. This is because sexually transmitted infections may be present without a person knowing or having symptoms.
Natsal-3 approach

- Voluntary anonymised testing
- Results not returned
- Specific informed written consent
- Information provided on access to STI advice, testing and treatment services
- Biological data anonymised
Natsal-3 approach

• Agreed in discussions between Natsal-3 team, study advisory group and other stakeholders
• Agreed by peer review in funding process
• Approach approved by research ethics committee
• Published in Journal of Medical Ethics (2011)

Testing for sexually transmitted infections in a population-based sexual health survey: development of an acceptable ethical approach

Nigel Field,1 Clare Tanton,1 Catherine H Mercer,1 Soazig Nicholson,2 Kate Soldan,3 Simon Beddows,3 Catherine Ison,3 Anne M Johnson,1 Pam Sonnenberg1
Natsal-3 approach in practice

• Interviewer training
• Piloting – supported acceptability
  – 61% (68/111) agreement rate
  – Reasons for refusal not linked to non-return of results

“Embarrassed”
“Not comfortable providing sample to a stranger”
“Did not have time”
“No particular reason”
Natsal-3 approach in practice

• Interviewer training

• Piloting – supported acceptability
  – 61% agreement rate
  – *Reasons for refusal not linked to non-return of results*

• Borne out in main survey
  – 60% agreement rate
  – *Similar reasons for refusal*
Recommendations

• Consider clinical implications
• Clinical accuracy of tests
• Context: how easy for participants to get tested in healthcare setting?
• Clear messages for participants
• Interviewer training
• Piloting
Acknowledgements

With thanks to:

• Natsal-3 participants
• NatCen Social Research Interviewers, Operations and Computing staff
• Natsal-3 advisory group

Natsal-3 was funded through grants from the Medical Research Council and Wellcome Trust with contributions from the Economic and Social Research Council and the Department of Health (England)
Thank you for listening

Questions / discussion