

Comparison of 3 Vitamin D Tests

Material and Methods: We conducted Vitamin D testing on 26 members of the public in the city of Galway using 3 different Vitamin D (25-hydroxyvitamin D) tests. The tests were supplied by Nutricentric Healthcare Ltd of Enniskerry, Co. Wicklow and Affimedix Inc. of California.

They were

- Rapi-D: A new semi-quantitative and quantitative test for 25(OH) Vitamin D. Rapi-D can be evaluated visually to give a semi-quantitative result or using an electronic reader (RapiRead) for calibrated quantitative results. In this case we rated results visually.
- Test 4D: A qualitative yes/no answer to the question, is Vitamin D status deficient (with the cutoff defined as <32ng/ml)
- LC-MS/MS blood spot testing. The spots were sent to a UK hospital laboratory (Sandwell and West Birmingham Hospitals NHS trust) for evaluation

The volunteers were recruited by advertising in a local Galway newspaper. The aim was to compare the methods and to gather some broader information on factors affecting Vitamin D status amongst these random volunteers. Data was collected (via questionnaire) about nutritional habits, sun exposure and supplement use. Each volunteer also provided written informed consent.

This current evaluation is restricted to comparison of the test results.

Testing was carried out in our Abbeygate Health Store, Galway City on July 19th and 20th and involved a total of 4 testers/readers over the course of the day. The tests were run in parallel and timed via an electronic timer App (Watson). To avoid bias, the Test4D and Rapi-D results were assessed by different evaluators.

All three tests were simple to carry out, though not all volunteers were willing to provide more than one sample. All 26 volunteers all were evaluated using Rapi-D, 25 of them using Test4D and 10 for 10 for LC-MDS/MS blood spot testing. Ultimately, only 9 of the blood spot tests could be evaluated.

The fingerprick samples were collected and reagent added in accordance with the instructions provided by the respective manufacturers.

Test4D results are evaluated at 10 minutes. Presence of one line in the test cassette denotes a Sufficient score of 32ng/ml or above (equivalent to 80 nmol/L) evaluated via presence or absence of a two lines. One line denotes Sufficient and 2 lines denote Deficient.

Results

For better comparison of the methods, we deemed results of 30ng/ml (75 nmol/L) and above to be Sufficient, results between 10 and 30ng/ml to be Insufficient and results below 10ng/ml to be Deficient. The results were unchanged even if the Test4D cutoff of 32ng/ml was used.

Test4D: Of the 3 tests used, Test4D gave the most uniform result. Every sample tested (100%) was determined to be Deficient (<32ng/ml). This result was in marked contrast to the results with Rapi-D and LC-MS/MS.

LC-MS/MSL: Three of the 9 LC-MS/MS samples (33%) were deemed to have Sufficient Vitamin D levels when 30ng/ml (75 nmol/L) was used as cut-off. The remainder were Insufficient (between 10 and 30ng/ml). The lab itself uses a lower classification score and considers results of 20ng/ml (50 nmol/L) as "Adequate". Thus cut-off was in widespread international use until recently. As a result, the hospital rated all but one of the samples as Adequate (89%).

Rapi-D: 7 of 26 samples (27%) were deemed to have Sufficient levels - denoting 25(OH) vitamin D of 30ng/ml (75nmol/L) and above. The remaining 19 (73%) were Insufficient, meaning that Vitamin D was below 30ng/ml (equivalent to 75nmol/L) but above 10ng/ml (equivalent to 25 nmol/L).

Both the Rapi-D and LC-MS/MS results found no samples that were classifiable as Deficient (< 10ng/ml or < 25 nmol/L).

Table 1: Test results using 3 test methods

#	Test4D	RapiD	NHS Rating
	Qualitative yes/no	Semi-Quantitative Reading	Quantitative LC-MS/MS
	DEFICIENT <32ng/ml <i>(equivalent to <80nmol/L)</i>	DEFICIENT <10ng/ml SUFFICIENT >30ng/ml <i>(25 & 75nmol/L respectively)</i>	DEFICIENT <10ng/ml SUFFICIENT >30 ng/ml <i>(25 & 75nmol/L respectively)*</i>
1	Deficient	Insufficient	Insufficient
2	Deficient	Insufficient	Insufficient
3	Deficient	Insufficient	
4	Deficient	Insufficient	Insufficient
5	Deficient	Insufficient	Insufficient
6	Deficient	Insufficient	Insufficient
7	Deficient	Insufficient	Sufficient
8	Deficient	Insufficient	
9	Deficient	Insufficient	
10	Deficient	Insufficient	
11	Deficient	Insufficient	
12	n/a	Sufficient	
13	Deficient	Insufficient	
14	Deficient	Insufficient	
15	Deficient	Insufficient	
16	Deficient	Insufficient	Insufficient
17	Deficient	Insufficient	
18	Deficient	Sufficient	
19	Deficient	Sufficient	
20	Deficient	Sufficient	Sufficient
21	Deficient	Sufficient	
22	Deficient	Sufficient	
23	Deficient	Insufficient	
24	Deficient	Sufficient	Sufficient
26	Deficient	Insufficient	

- NHS Laboratory definition of Adequate differed and was set at 50nmol/L (20ng/ml)

Discussion

Both Test4D and Rapi-D were instant Point of Care tests - yielding results within 10 and 15 minutes respectively. The lab test results – currently the gold standard - took an average of 2 weeks to arrive by e-mail.

The tests were easy to conduct. Of the two “instant” tests, Rapi-D provided more nuanced and credible results than Test4D, which rated all samples as “Deficient”

While studies of Vitamin D status in Ireland tend to show a significant minority of Deficient results (upwards of 10% in summer¹), there are a few factors suggesting greater credibility for the Rapi-D and lab results.

First amongst these is the timing of the study. The early part of summer 2017 was sunnier than usual in Galway – as evidenced on data from the meteorological station in Athenry, Co. Galway.

Global Solar Radiation in Joules/cm² for Athenry, Co. Galway

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2017	8075	11153	27039	33778	58096	49741	48651	40221	17740				294494
2016	6395	12451	26983	40881	54301	51875	43449	35597	23011	17607	10166	5866	328582
2015	7492	11565	28019	48205	48078	54373	45987	42658	29742	17541	7337	3784	344781
2014	7182	12668	25023	42001	43382	53399	47995	42454	32446	17405	9215	5407	338577

The second factor is the general finding that older people, with more time to spend in the sun, tend to have better Vitamin D levels¹. Of the 26 people in our study, only 15 disclosed their age. Of these, 12 were aged 50 and above, 7 were aged 60 and above and 3 were above 70.

Conclusions:

Rapi-D semi-quantitative evaluation provided rapid actionable results that agreed well with samples evaluated by the gold-standard laboratory method. Test4D provided questionable results drawn from the same population. Despite good sunny weather in the 3 preceding months, 73% and 67% of volunteers assessed by Rapi-D and LC-MS/MS respectively had Insufficient Vitamin D levels (below 30ng/ml (75 nmol/L).

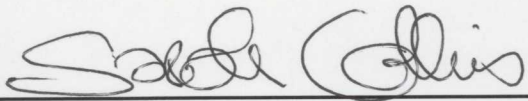
References

1. E. Laird, T. Shannon, V. E. F. Crowley, M. Healy 'The benefits of utilising geo-mapping for visualising the vitamin D status of Dublin city and the surrounding urban districts' Ir J Med Sci (2016). <https://doi.org/10.1007/s11845-016-1517-42>
2. Meteorological data sourced from <https://www.met.ie/climate/monthly-data.asp?Num=1875>

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Signed:



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