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Should tumbling E go out of date in amblyopia screening? Evidence from a population-based sample normative in children aged 3–4 years

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ABSTRACT

Aims To determine a normative of tumbling E optotype and its feasibility for visual acuity (VA) assessment in children aged 3-4 years.

Methods A cross-sectional study of 1756 children who were invited to participate in a comprehensive non-invasive eye exam. Uncorrected monocular VA with crowded tumbling E with a comprehensive ophthalmological examination were assessed. Testability rates of the whole population and VA of the healthy children for different age subgroups, gender, school type and the order of testing in which the ophthalmological examination was performed were evaluated.

Results The overall testability rate was 95% (92% and 98% for children aged 3 and 4 years, respectively). The mean VA of the first-day assessment (first-VA) and best-VA over 2 days' assessments was 0.14 logMAR (95% CI 0.14 to 0.15) (decimal=0.72, 95% CI 0.71 to 0.73) and 0.13 logMAR (95% CI 0.13 to 0.14) (decimal=0.74, 95% CI 0.73 to 0.74). Analysis with age showed differences between groups in first-VA ($F(3,1146)=10.0$; $p<0.001$; $\eta 2=0.026$) and best-VA ($F(3,1155)=8.8$; $p<0.001$; $\eta 2=0.022$). Our normative was very highly correlated with previous reported HOTV-Amblyopia-Treatment-Study (HOTV-ATS) (first-VA, r=0.97; best-VA, r=0.99), with 0.8 to 0.7 lines consistent overestimation for HOTV-ATS as described in literature. Overall false-positive referral was 1.3%, being specially low regarding anisometropias of ≥2 logMAR lines (0.17%). Interocular difference ≥1 line VA logMAR was not associated with age (p=0.195).

Conclusions This is the first normative for European Caucasian children with single crowded tumbling E in healthy eyes and the largest study comparing 3 and 4 years old testability. Testability rates are higher than found in literature with other optotypes, especially in children aged 3 years, where we found 5%–11% better testability rates.

INTRODUCTION

Although WHO and the National Academy of Sciences Committee on Vision (NASCV) have issued recommendations about the design of optotypes used in visual acuity (VA) charts that were included in international guidelines,[1] several charts used with children meet these criteria: LEA symbols, Sloan letters/numbers, tumbling E and HOTV. Although HOTV test is commonly used in the USA,[2–4] no standard VA testing in children has been accepted worldwide.[5 6] Directional optotypes as Landolt C and tumbling E charts are widely used in non-spoken English countries.[5 7] They are considered better optotypes as grating recognition and orientation is superior to letter recognition[8] and the progression of resolution angles is similar throughout the test.[9] Tumbling E has the advantage of performing better than Landolt C for astigmatism against-rule.[10] Test–retest tumbling E studies demonstrated highly repeatable results (r=0.97).[11] Nevertheless, tumbling E has been reported as conceptually difficult for young children[1] as it requires spatial orientation skills, and recently, it has been non-recommended in young children as *'they may not yet have developed the ability to express the orientation of these optotypes'*.[4] Doubts whether up-down-right-left orientation is already mastered by children aged 3 years old (yo), or not, is doubtful.

Although single optotypes have better cooperation in children if crowding is not used,[4 12] crowded bars are more accurate[4 13] to overcome the 'crowding phenomenon' present in amblyopia and should be used.[14] In amblyopic children, the kind of crowded optotype also revealed differences depending on the optotype.[15] Normative with single crowded HOTV[2 3 16] and LEA[12] have been published. Regarding tumbling E, normative are either in charts,[5 7 17] lines[18] or single E without crowded bars,[18] but to the best of our knowledge, no normative with single-crowded tumbling E is available in the optimal age screening,[19] making it crucial to set a normative.

Normal VA in healthy eyes should be strictly assessed when setting a normative with accurate referral criteria for vision screening. It must be ensured that all ophthalmological pathologies are excluded. Some recent normative had these important considerations into account,[3 12 16 17] but none with single-crowded tumbling E.

The scope of this study is to determine a normative for 3–4 yo in healthy eyes with tumbling E in the single-crowded form and to determine if it is a feasible VA tool in this age group. We search for differences between 3 and 4 yo in testability, false-positive referrals and we compare it with other previous normative published. A normative regarding age will be presented.

MATERIALS AND METHODS

Participants

Between May 2014 and June 2016, all children registered in Portuguese National Health Service with 3–4 yo, attending public and private schools in

Braga, were invited to participate in an eye exam at the Ophthalmology Department of the *Hospital de Braga*. This study was approved by the Human Research Ethics Committee of the *Hospital de Braga* and adhered to the tenets of Declaration of Helsinki. Written informed consent was obtained from the parent/guardian of child before examinations.

Procedures, ophthalmological examination and VA assessment

All children underwent a comprehensive non-invasive eye exam performed by orthoptists and paediatric ophthalmologists trained in the study's protocol. Age, sex, school source and order of testing were registered. Examination included uncorrected monocular VA with single-crowded tumbling E (VA assessment was always done by experienced orthoptists in children examination), ocular motility, cover test, Hirshberg, three Plusoptix S04 measures, Randot stereoacuity with circles, biomicroscopy and funduscopy. All astigmatisms were registered in negative cylinders for comparison proposes.

VA was recorded as the smallest optotype size which the child identified in all four directions, in order not to misdiagnose meridional amblyopia. VA scores were provided in 0.1 decimal increments, recorded in a decimal scale and converted to logMAR for analysis. After a brief training with binocular VA, testing was conducted monocularly, always starting with the right eye (OD). The eye was occluded with a non-adhesive paper occluder, explaining the child it was a 'pirate game'. The child could either use fingers or a plastic E to indicate the direction of the letter. Half of the children started the exam with VA measurement and then went to perform other exams (VA was assessed in the end in the remainder). These VA results were considered the first visual acuity assessment (first-VA) as some children were called for a second evaluation as explained ahead. For testability rates, a child was considered testable if it was possible to determine monocular first-VA in both eyes. Stereoacuity measurements were done with Randot test. Emetropia was defined as: normal spherical equivalent (SE) based on 98.6% negative predictive value (NPV) for hyperopia adapted criteria (SE <2.0 dioptres) and 98% NPV for astigmatism (<1.5 dioptres) for Plusoptix.[20] Myopia and anisometropia criteria were SE <−0.5 dioptres and sphere or cylinder <1.0 difference, respectively. We applied these criteria for all three consecutive Plusoptix measurements.

If emetropic children with no associated ophthalmological pathology had poor collaboration or a monocular first-VA without correction ≤0.6 or interocular difference ≥3 lines in decimal scale, children's VA was reassessed on a second day, this time in their parent's presence. In the second VA assessment, the same protocol was applied. We considered the best VA (best-VA) over the first and second evaluation only for comparison purposes with other recent normative published.[16] Nevertheless, we used first-VA as our main normative outcome variable because it best reflects screening practice and, besides, using the best of multiple measures of VA could introduce a systematic bias, causing false conclusions.[21]

Exclusion/inclusion criteria

Only children aged between 36 and 59 months were included. After excluding 12 children that came twice to the exam, the whole population of 1735 was considered for testability assessment. For VA normative, only healthy eyes were considered and exclusion criteria are presented in figure 1.

STATISTICAL ANALYSIS

Data were gathered in Microsoft Office Excel and exported to IBM Statistical Package for the Social Sciences Statistics (SPSS V.24) for statistical analysis. VA was measured in a decimal scale and converted into logMAR units for statistical analyses as recommended.[22] For being more comprehensive, VA means, differences of means, SD and CIs were also expressed in decimal values right after all logMAR results (into parenthesis). The statistical procedures assumptions, such as normality, homogeneity of variances, were considered. When appropriate, Student's paired-sample t-test, independent-sample t-test, one-way analysis of variance or Welch test were used whether there was homogeneity of variances or not in Levene's test, and the corresponding post hoc testing was done, either with Bonferroni or Games-Howell, if there was statistical significance. When Levene's test indicated unequal variances, df was adjusted correspondingly. Effect size measures along with statistical significance ($p<0.05$) were presented.

RESULTS

Participation rate of the invited children was 96%. Regarding testability, we found 89/1735 (5.1%) non-cooperative children in VA responses in at least one eye. Testability in age subgroups is presented in table 1.

Testability in different age subgroups of 3.0 (36–41 months), 3.5 (42–47 months), 4.0 (48–53 months) and 4.5 (54–59 months) yo. On the right, comparison between 3 and 4 yo.

Of the 1182 children that met the criteria for sample VA normative (figure 1), median age was 47.37±4.74 months, 51.0% were males, 54% attended public schools and in 43% ophthalmological examination started with VA assessment. The mean first-VA was 0.14 logMAR (95% CI 0.14 to 0.15) (0.72 (95% CI 0.71 to 0.73)) for OD and 0.14 logMAR (95% CI 0.14 to 0.15) (0.72, (95% CI 0.71 to 0.73) for OS. Normality of these measures where proven by acceptable values of kurtosis and skewness (OD: 0.75; 0.44/OS: 0.94; 0.49) and histogram distribution. As first-VA for the OD and OS were not significantly different ($t(1149)=-0.22$, $p=0.826$, $d=0.00646$, paired sample t-test), ($r(1150)=0.67$, $p<0.001$, Pearson's correlation), we used first-VA OD as the normative of monocular VA in the first observation. Differences ≥1 line VA logMAR was found in 116 children (9.8%).

From the 157 with at least one criteria for a second evaluation, 94 were re-examined on a second day, for VA re-assessment (participation rate 60%). Differences between the best-VA OD and OS were not significant ($t(1158)=1.14$, $p=0.254$, $d=0.0335$) ($r(1159)=0.62$, $p<0.001$). From now on, we will no longer report OD or OS, as we will always report OD results, either regarding first-VA or best-VA, unless specified differently.

First-VA and best-VA had no differences regarding gender and the order in which the ophthalmological examination was performed, respectively, ($t(1148)=0.428$, $p=0.668$, $d=0.026$) and ($t(558)=0.126$, $p=0.90$, $d=0.010$) for first-VA, and ($t(1157)=0.916$, $p=0.36$, $d=0.054$) and ($t(565)=1.13$, $p=0.261$, $d=0.097$) for best-VA. Regarding school type, there was a difference of 0.014 logMAR (lower logMAR for public schools) found in first-VA ($t(1030.7)=-2.85$, $p=0.005$, $d=0.17$).

Regarding first-VA, age had a very small correlation ($r(1150)=-0.17$, $p<0.001$) and differences found between 3 and 4 yo were 0.022 logMAR (95% CI 0.012 to 0.031) ($t(1135.2)=4.6$, $p<0.001$, $d=0.27$, independent sample t-test). With best-VA, similar differences of 0.018 logMAR were

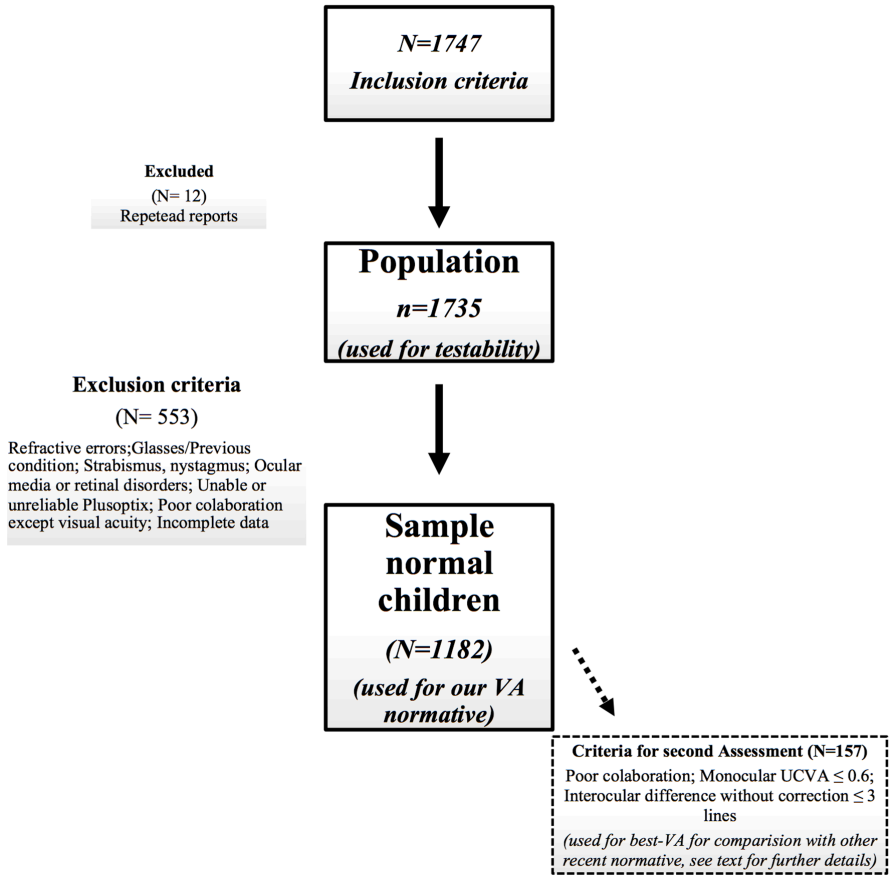

Figure 1 Inclusion/exclusion criteria for testability rates and for visual acuity (VA) normative. The interocular difference was measured in decimal lines. UCVA, uncorrected visual acuity.

observed (95% CI 0.001 to 0.027) (t(1150.4)=4.2, p<0.001, d=0.25, independent sample t-test). For comparison with recent normative studies, we present normative of first-VA and best-VA in four age subgroups in table 2.

Analysis showed differences between groups in first-VA (F(3,1146)=10.0; p<0.001; η2=0.026) and best-VA (F(3,1155)=8.8; p<0.001; η2=0.022). Post hoc analysis using Bonferroni test showed no difference in first-VA between 3.0 and 3.5 yo (p=0.83). All other subgroups had statistically significant differences of 0.01556 logMAR (95% CI 0.0018 to 0.0293), p=0.019 between 3.5 and 4.0 yo, 0.02274 logMAR (95% CI 0.0011 to 0.0444), p=0.036 between 3.0 and 4.0 yo, 0.03906 logMAR (95% CI 0.0193 to 0.0588), p<0.001 between 3.5 and 4.5 yo, 0.04624 logMAR (95% CI 0.0204 to 0.0721), p<0.001 between 3.0 and 4.5 yo, 0.02350 logMAR (95% CI 0.0042 to 0.0428), p=0.010 between 4.0 and 4.5 yo.

Regarding false-positive referrals, for children aged between 36 and 47 months, with the recommended cut-off value of 0.4 logMAR (0.4), we had 1% of false-positive referral. For children aged between 48 and 59 months, for the recommended cut-off value of 0.3 logMAR (0.5), we had 1.5% of false-positive referral. For false-positive referrals regarding anisometropias, we used a difference ≥2 logMAR lines between OD and OS as the cut-off value. Of the 1182 children, only two had anisometropia. We used the second evaluation result as the true diagnosis and none of those two had in fact anisometropia, giving a false-positive referral of 0.17%.

Table 1 Testability

Age		n		Non-cooperative		Testability (%)	
3.0	3 yo	233	929	36	75	84.5	91.9
3.5		696		39		94.3	
4.0	4 yo	638	806	14	14	97.8	98.3
4.5		168		0		100.0	
Total			1735		89		94.9

yo, year old.

DISCUSSION

This is the first population-based normative for European Caucasian children with single-crowded tumbling E. It is also the largest study comparing 3 and 4 yo testability, such an important age range for screening purposes.

Our exclusion criteria are similar to others[3 16 17] that were also judicious about measuring VA in only healthy eyes. Based on refractive criteria, we excluded 20.4% children, in line with Leone *et al*[16] that excluded 29.9% but in a wider age range (6–72 months), where more refractive errors are expected to be found.

Although we have higher global testability rates (94.9%), our main advantage is in the 3 years' group, where our testability is considerably higher (table 3).

Although our global testability was lower than the 97.6% reported with HOTV-ATS,[23] it is important to note that higher testability reported by Kupl *et al* could have been achieved by older children, as 28% of children in that study were 5 yo, and children between 3.0 and 3.5 yo were not included. If we compare similar age groups, we find 94.3% and 98.3% testability, better than 93.3% and 96.7% at 3.5 and 4 yo respectively, found

Table 2 Normative for the first-VA and the best-VA

	First-VA			Best-VA		
Age yo (months)	n	LogMAR (95% CI)	Decimal (95% CI)	n	LogMAR (95% CI)	Decimal (95% CI)
3 yo (36–41)	118	0.15 (0.14 to 0.17)	0.70 (0.67 to 0.72)	120	0.15 (0.14 to 0.17)	0.70 (0.68 to 0.73)
3.5 yo (42–47)	453	0.15 (0.14 to 0.16)	0.71 (0.69 to 0.72)	459	0.14 (0.13 to 0.15)	0.73 (0.71 to 0.74)
4 yo (48–53)	464	0.14 (0.13 to 0.14)	0.73 (0.72 to 0.74)	465	0.13 (0.12 to 0.13)	0.74 (0.73 to 0.76)
4.5 yo (54–59)	115	0.11 (0.10 to 0.12)	0.77 (0.75 to 0.80)	115	0.11 (0.10 to 0.12)	0.78 (0.75 to 0.80)

VA of the first-VA and of the best-VA over two assessments; yo, years old; VA, visual acuity.

with HOTV-ATS.[23] Comparing our testability with HOTV-ATS in four age subgroups described in 2012,[24] our rates are 4.5%, 1.3%, 2.8% and 2% higher than HOTV-ATS for 3.0, 3.5, 4.0 and 4.5 yo, respectively, also showing the best benefit for 3.0 yo.

Regarding testability rates, one strong point in our study is that it reflects testability of all children before exclusion criteria, meaning that testability among children with refractive errors and/or amblyopia is also considered. If we had considered only normal eyes in our study, the overall testability rate would had been 97.3%.

We speculate that this good testability scores could be achieved because today's children stimulation with Smartphones and Tablets at very young ages leads to up-down-left-right recognition ability developed sooner and the exam could be experienced as another game. Besides, we realised that children tend to cooperate much better if they are 'in-group' with schoolteachers and colleges than when they come alone with their parents. It happens as if the child understands better his/her role of commitment to the performance of the tests, seeing it as a school extension. As a limitation, our better testability could be due to the population studied, as Braga is a modern urban city.

We used first-VA for the normative as it best reflects clinical practice. Regarding VA variation with age, there is very small intensity of the negative correlation found. Moreover, either in first-VA or best-VA age subgroups variation analyses, the biggest difference found was between 3.0 and 4.5 yo, but differences found between all age subgroups were <0.5 logMAR lines, what, in our opinion is not clinically relevant, as the interval measurements used in logMAR VA scales are 0.1 logMAR units (1 line), making differences of <0.05 logMAR units (<0.5 lines) below the threshold scale measurement, so, not possible to detect in clinical practice and, for the same reason, would not change VA thresholds normative guidelines. Effect size of these differences analyses revealed that age only explained 2.6% and 2.2% of first-VA and best-VA, respectively. Regarding interocular differences of ≥1 line VA logMAR, we had only 9.8%, <26% report[16] and it was not associated with age (p=0.195) as reported earlier.[16]

Comparing our best-VA normative (table 4) with the recently reported HOTV-ATS,[16] we found HOTV-ATS has 0.079 and 0.072 logMAR consistently better VA than our first-VA and best-VA with very high correlations (r=0.97 and r=0.99), respectively. It is important to note that this HOTV-ATS normative is based on the best-VA of two consecutive measures in two different appointments, what is the equivalent as our best-VA. The same tendency is described in literature regarding HOTV

Table 3 Testability rates with different optotypes

		n		Testability	
		3 yo		3 yo	
Study	Optotype	4 yo	total	4 yo	total
Present study	Single-crowded tumbling E	929	1735	91.90%	94.9%
		806		98.30%	
Leone *et al*[24]	HOTV-ATS	363	723	86.5%	91.4%
		360		96.3%	
Pan *et al*[3]	HOTV-ATS	460	1027	81%	90.5%
		567		98%	
Kvarnstrom *et al*[27]	HOTV chart	478	707	84.80%	87.4%
		229		92.80%	
Kvarnstrom *et al*[27]	LEA chart	478	707	82.80%	86.1%
		229		92.90%	
Kupl *et al*[23]	HOTV-ATS	225	858	*(3–3.5 yo not included)*	*97.6% (5yo included)*
		633		96.70%	
Becker *et al*[12] *Paediatric routine examination*	Single LEA symbols	71	134	76.00%	85.00%
		63		95.00%	
Becker *et al*[12] *Ophthalmic revaluation*	Single LEA symbols	10	28	80.00%	92.80%
		18		100.00%	

For a more accurate comparison, testability rates are presented separately at 3 and 4 yo.
yo, years old.

Table 4 Normative of tumbling E and HOTV

Study	Optotype	Mean logMAR best-VA (95% CI) *decimal*			
		3.0 yo	3.5 yo	4.0 yo	4.5 yo
Present study	Single-crowded tumbling |E|	0.15 (0.14 to 0.17) *(0.70 (0.68 to 0.73))*	0.14 (0.13 to 0.15) *(0.73 (0.71 to 0.74))*	0.13 (0.12 to 0.13) *(0.74 (0.73 to 0.76))*	0.11 (0.10 to 0.12) *(0.78 (0.75 to 0.80))*
Leone *et al*[16]	HOTV-ATS	0.09 (0.07 to 0.10) *(0.81 (0.79 to 0.85))*	0.07 (0.05 to 0.09) *(0.85 (0.81 to 0.89))*	0.05 (0.03 to 0.06) *(0.89 (0.87 to 0.93))*	0.03 (0.02 to 0.04) *(0.93 (0.91 to 0.95))*

VA, visual acuity.

charts and ETDRS charts, where HOTV-ATS has approximately 1 line better VA results.[24]

Comparing our best-VA normative with HOTV in different age subgroups of 3.0 (36–41 months), 3.5 (42–47 months), 4.0 (48–53 months) and 4.5 (54–59 months) yo. Note that single crowded optotypes and best-VA over two observations are use in both studies.

Despite the limitation of this assumption, if we consider school type as a proxy of socioeconomic status, the difference of 0.014 logMAR found in first-VA, although statistically significant, had a small effect size, and to our interpretation it should not be considered relevant.[25]

Regarding false-positive referrals, for 3 yo we had 1% of false-positive referral, which is similar to the 0.9% found by Leone *et al*,[16] using the same cut-offs, with HOTV-ATS protocol[26] on the electronic VA tester and better than the 5.3% they found with ETDRS or HOTV chart.[16] For children 4 yo, we had 1.5% false-positive referrals, which is 1.1% higher than the 0.4% found by Leone *et al* with HOTV-ATS[26] and much lower than the 6% found with ETDRS or HOTV chart.[16] Regarding false-positive referrals for anisometropias, we had a very low false-positive referral of 0.17%, much lower than 6% described by Pan *et al* with HOTV-ATS.[3] Their normative, nevertheless represents a wider age range (30–72 months).

As limitations, we only studied Braga's population. We did not use race as a variable because being our population mainly Caucasian, we assume it reflects no influence in our results. In a 235 sample, we only reported two non–Caucasians (0.85%). The time of the exam was not assessed. The published differences of 1.12 more minutes to test a 3 yo child vs 4 yo child with LEA and HOTV charts,[27] arises the question as if these differences are relevant, considering the time spent for receiving the child, register the results and giving the report of the screening. Further studies are needed regarding this issue, including time to perform the exams in healthy versus unhealthy eyes.

In summary, tumbling E has the highest testability rates between 3 and 4 yo when compared with literature, specially in children 3 yo where it is considerably better. Past evidences fearing the differences in collaboration between 3 and 4 yo are no longer supported. In screenings where VA is the gold standard, tumbling E is a good instrument for VA assessment in children aged 3 and 4 years.

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Contributors Research design, data analysis and interpretation: SG, PC and ES. Data acquisition and research execution: SG and TF. All authors participated in manuscript preparation.

Competing interests None declared.

Patient consent All children aged between 3 and 4 years from public and private schools in Braga, Portugal, were invited to participate in an eye exam at the Ophthalmology Department of the Hospital de Braga. All children were legal residents, registered in Portuguese National Health Service. Written informed consent was obtained from the parent or guardian of each child before examinations. The consent was not a specific consent from BMJ. The written consent was approved by the Human Research Ethics Committee of the Hospital de Braga and adhered to the tenets of the Declaration of Helsinki.

Ethics approval Human Research Ethics Committee of the Hospital de Braga.

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